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ALTERNATIVES TO ANIMAL USE IN RESEARACH, TESTING, AND EDUCATION

Contractor Documents

Volume I: Overview

February 1986

"Survey and Estimates of Laboratory Animal Usage in the United States," Kurt Enslein, Health Designs, Inc., Rochester, N.Y.

"Ethical Considerations," Arthur H. Flemming, Department of Philosophy, The University of Chicago, Chicago, Ill.

"Scope of 'Alternatives': Overview of the State of the Art," Roland M. Nardone and Lucille A. Ouelette, Department of Biology, Catholic University, Washington, D.C.

"Overview of Computer Use in Research, Testing and Education," Paul N. Craig, Shadyside, Md.

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Survey and Estimates of Laboratory Animals Usage in the U.S.

Report Prepared for the
Office of Technology Assessment,
U.S. Congress

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TABLE OF CONTENTS

Page

0. EXECUTIVE SUMMARY 1

I. INTRODUCTION 4

- A. Purposes of this Report 4
  - 1. To estimate current dimensions of laboratory animal use in the United States
  - 2. To project, if possible, future use of laboratory animals in this country
- B. Overview 4
  - 1. Collection of all publicly-available information
  - 2. Critical evaluation of these data, including assessments of their
    - a) Reliability for the tasks of estimation and projection of animal use, and
    - b) Limitations for tasks of estimation and projection of animal use.
  - 3. Collection of the most reliable data on animal use
    - a) ILAR
    - b) APHIS
  - 4. Estimate and projected use of laboratory animals using
    - a) collected data (ILAR and APHIS)
    - b) more indirect means (based on NIH funding, NCI animal usage, and NIH total usage as a function of intramural use)
- C. Limitations of this approach, implications of these limitations, and suggestions for improved study 5
  - 1. Limitations:
    - a) Extrinsic to study:
      - (1) Problems with the data sources
    - b). Intrinsic to study
      - (1) Shortage of resources
  - 2. Implications of limitations
  - 3. Suggestions for improved study
    - a) Stratified random sample survey

II. SUMMARY OF INTEGRATED DATA 6

A. Introduction 6

B. Tables 8

III. CRITICAL EVALUATION OF SOURCES OF SUMMARIZED DATA 19

A. Introduction 19

B. Evaluation of Sources 20

1. ILAR Surveys
2. USDA APHIS Anual Reports and Institutional Data Sheets
3. Saunders and Company
4. MCA Study
5. Alex Brown & Sons (1981) Study
6. Amphibians
7. Fish
8. Birds

IV. ESTIMATION OF ANNUAL LABORATORY ANIMAL USE 31

A. Estimation Using Indirect Means 31
1. Based on NIH funding
2. Based on NCI usage
3. Based on NIH total usage as a function of intramural use

B. Estimation Using Corrected Data from ILAR 1978 36
1. Method (a)
2. Method (b)
3. Method (c)

C. Estimation Using Corrected Data for APHIS summary reports for FY 1982 and FY 1983 38
1. Rats and Mice
2. Mandated species
3. Other species
4. Total

V. **ANALYSIS OF ESTIMATIONS AND CONCLUSIONS (including recommendations for future study)** 45

  A. General Profile 45

  B. Number of Reporting Institutions 46

  C. Changes of Significance within Specific Types of Surveys 47

  D. General Comments Regarding Comparison of Data from Different Types of Surveys 47

  E. Trends and Projections of Future Laboratory Animal Use 47

  F. Recommendations for Future Study 49

VI. **REFERENCES** 50

  A. Primary Sources 50

  B. Secondary Sources 52

  C. Resource Persons 54

VII. **APPENDIXES**

A. Costs - Rat and Mouse

B. Regression Equations

C. Listing of 1982 and 1983 APHIS Data

0. EXECUTIVE SUMMARY

The purpose of this project was to study existing reports and other sources of data relating to the number of laboratory animals used in the United States, and from these, and other information, estimate current and projected usage of the five classes of vertebrates.

The data sources consisted mostly of the various reports and surveys published by the Institute of Laboratory Animal Resources (ILAR) of the National Academy of Sciences, various market surveys, several books dealing with the topic of laboratory animal usage, and the Annual Reports published by the United States Department of Agriculture/Animal and Plant Health Inspection Service (APHIS). For the latter source, the individual annual reports furnished by each registered facility were used as the basis of the evaluation.

Generally, it was found that great disparities exist between the numbers quoted in different sources. A critical evaluation of the sources showed that, with one or two exceptions, the methodology was uncertain enough that only little confidence could be put in the resulting numbers.

It was found that the most useful source of data, both for retrospective, current and, perhaps, projected future use were the APHIS data sheets, filled out by every institution which uses laboratory animals. These were compiled for fiscal years 1982 and 1983. APHIS requires that the registered institutions report the use of dogs, cats, guinea pigs, hamsters, rabbits and non-human primates. Reporting of other species is voluntary, and indeed, many different species are reported by many institutions. For the fiscal years 1982 and 1983, most institutions received forms which also listed rats and mice, though there was no requirement that those lines be filled out. About two-thirds of the institutions did report the use of rats and mice, and, consequently, these data could be used to estimate mouse and rat usage for those institutions not reporting, by means of two alternative statistical techniques.

The usage of the major species in 1982 and 1983 are shown below:

	FY 1982	FY 1983
Rats	3,400,000	3,700,000
Mice	8,500,000	8,500,000
Dogs	194,867	182,425
Cats	59,961	55,346
Guinea Pigs	497,860	521,237
Hamsters	417,267	454,479
Rabbits	547,312	509,052
Primates, non-human	54,565	59,336

Only relatively soft numbers could be obtained for amphibians, fish and birds. For these species, the APHIS reports were not particularly useful. The annual estimated use of these species is shown below:

Amphibians	100,000 - 500,000/year
Fish ½" or larger	500,000 - 1,000,000/year
less than ½"	2 million - 3 million/year
Birds	25,000 - 100,000/year

The largest users of laboratory animals are medical schools and universities, biomedical research organizations, pharmaceutical companies and federal institutions. Toxicological laboratories use relatively few lab animals.

From the comparisons of 1982 and 1983, it is not clear that many trends in lab animal usage exist. The data available from other than the APHIS reports is such that comparisons for other years cannot be made for rats and mice. For the APHIS-mandated species, no clear trends over time were found either, partly, it is believed, due to the fact that 10-20% of the individual institutional reports in each year are not used in the yearly APHIS compilations. Since the project dealt with data from the original institutional data sheets, this problem does not exist for the FY 1982 and 1983 data in this report.

When comparing previously reported numbers of lab animals used with the numbers in this report, it is well to bear in mind that many of the previous reports dealt with animals acquired, not used. Since it is generally believed that not more than half the animals bred are eventually used in lab experimentation, this fact alone accounts for a substantial part of the variance between numbers obtained from different sources.

The results of this study could have been considerably strengthened if it had not been for the severe financial limitations under which the study was carried out. Better trends for the APHIS-mandated species could have been obtained if data for these species for earlier years could also have been analyzed. More accurate estimates for rats and mice could have been obtained if more effort could have been devoted to the estimating methods, particularly in the examination of some of the unusual values. It would also have been possible to obtain extrapolations for mice and rats for each type of institution. But most importantly, the statistical design of the entire study really should have been a stratified random sample of all possible insititutions, so that greater certainty would have existed that some institutions were not being missed, and others as a result acquired too great an influence on the results.

I. INTRODUCTION

A. Purpose of This Report

The overall purposes of this report were to estimate the current dimensions of laboratory animal use in the United States, and project, if possible, future use of lab animals.

B. Overview

All publicly-available information on past and current animal use were collected from a variety of sources, often through personal contact. These data were then subjected to critical evaluation to determine the adequacy of the survey designs, data collection, etc., in order to make a judgment as to the usefulness and reliability of the data in reporting current usage, and for purposes of making projections of animal use.

Data from the two most reliable sources, i.e., the Institute of Laboratory Animal Resources (ILAR) of the National Academy of Sciences, and the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) were corrected to take into account the actual years of reporting, and omission of certain data that were not received before a data cutoff deadline.

Laboratory animal use was then estimated and projected using computer and statistical techniques where appropriate. For this purpose, the corrected ILAR and APHIS data were used, as well as more indirect means based on National Institutes of Health (NIH) funding, National Cancer Institute (NCI) usage, and NIH total usage as a function of NIH intramural use. While number of animals bred might be expected to lead to good estimates of animals used in the laboratory, the reliability of the production numbers was low enough that only poor estimates could be derived by their use.

C. Limitations of this Approach, Implications of these Limitations and Suggestions for Further Study

Two types of limitations exist: extrinsic to the study and intrinsic to the study. The extrinsic limitations include inadequacy of most of the survey and data collection methodologies, difficulties with definitions of use, area of use, reporting requirements and completeness. The major intrinsic limitations were a lack of adequate funding levels and a limited time span during which the study could be performed.

The main implications of these limitations reflect on the accuracy of the data and the projections based on them. The limitations were such that the only source of reasonable credibility for current use and projection to the future were the APHIS data, particularly the institutional data sheets. Due to limitations of funds and time, only the detailed APHIS institutional data sheets for FY 1982 and 1983 were used, though those for FY 1981 were also available (as could be those for other years as well, were the necessary resources available to process these data). Consequently, the reliability of the APHIS data are poorer for the years before 1982 inasmuch as these are based on the reports to Congress, which are known to underestimate use of the mandated species by 10 to 20% and do not treat data from Federal Institutions consistently. In other areas, such as fish and birds, only estimates of use could be obtained, in view of the diffuse nature of use. Again, these numbers could have been more accurate if further resources could have been devoted to their collection and analysis.

The study could be improved by two major approaches: the first, and least expensive, would involve the use of the remaining data sheets of APHIS, as well as thoroughly determining which of the registered instutions in each year did and did not report, so that appropriate statistical estimation techniques could be used on an institution type and year-specific basis to correct for the missing data. The second, and more ambitious approach, would be to conduct a stratified random sample study of all possible users. The stratification would be by type of institution, size of institution and species of animals. From such a sample, one could then make, again by the appropriate statistical techniques, projections to the entire population of user institutions.

II. SUMMARY OF INTEGRATED DATA

A. Introduction

The data tables shown in this section consist of a compilation of previously collected and, mostly, published data, arranged in such a way that they may be easily compared. Secondary references are not included, nor are "guesstimates" containing no substantiation (i.e., Homberger, 1976 or Santamarina, 1976).

In view of the fact that one of the objectives of the study was to be able to determine the number of animals used by various types of institutions, the following coding system was established:

Type #

1. Institutions of higher learning (beyond secondary), excepting medical schools.

2. Medical schools and colleges. This includes medical centers if associated with a university but not otherwise. Some medical schools are included among category one if the institution reports its laboratory animal use centrally.

3. Hospitals, clinics and medical centers not associated with an institution of higher learning. This category includes veterinary hospitals.

4. For-profit biomedical research institutions.

5. For-profit toxicology testing laboratories.

6. Not-for-profit biomedical research institutions.

7. For-profit chemical firms not including pharmaceutical subsidiaries of such firms. This category does include cosmetic firms and consumer products.

8. Pharmaceutical firms.

9. Devices and diagnostics firms.

10. Food and feed manufacturers.

11. Miscellaneous commercial firms not included in the above categories.

12. State and local governments.

13. Federal agencies including the Public Health Service (including the Food and Drug Administration and the National Institutes of Health), U.S. Army, Veterans Administration, other military, and the U.S. Department of Agriculture as well as miscellaneous federal institutions including environmental, natural resources, veterinary and marine research.

It will be noted that distinctions have been made between various types of research institutions; for example, since some are associated with for-profit and others non-profit institutions, the not-for-profit institution might be a university, which also has a medical school, etc. In order to be able to define the number of animals then, it was necessary to distribute the animals within an institution to the respective types of subinstitutions. This was frequently possible for the APHIS data, since a given institution would often have more than one registered facility. While the use of the broader testing, research and education categories could have been used, one would not have been able to distinguish for example, between toxicological testing labs and the toxicological testing performed by pharmaceutical companies. The toxicological testing labs perform much of their work for the pharmaceutical industry. Many not-for-profit so-called research institutions are actually testing laboratories, etc. It is also apparent that education would have to include universities as well as their medical schools, whereas the medical school needs are very different from the psychology department for example.

While the original institutional type assignment was made as explained above, it became evident that certain distinctions could not be made as well as desired without inquiry at each institution, and for this there was insufficient time. Consequently, the following categorizations were used for reporting purposes:

Type #	Types of Institutions
1,2	Universities
3	Hospitals, etc., not university affiliated
4,6	Biomedical research
5	Toxicological testing laboratories
7	Chemical companies
8,9	Pharmaceutical, devices and diagnostics companies
10,11	Food, feed and miscellaneous commercial firms
12	State and local governments
13	Federal agencies

B. Tables

In this section, we present tables which integrate results from various surveys. Table II-1 displays the data from the 1964, and 1965-71 ILAR surveys. One should bear in mind that the 1960 data (obtained from the 1964 report) was obtained from a subset of only 561 institutions.

Table II-2 shows the data from the ILAR 1980 report, which included both 1967 and 1978 data. One should point out that in the report proper, the 1967 data are referred to as year 1968.

Table II-3 shows the data from the USDA/APHIS Annual Reports for the mandated species. These are the data as reported to Congress, except that, where necessary and possible, some totals have beed adjusted to include data from Federal agencies. (See Tables II-4 and II-5 for corrected data based on institutional data sheets.)

Table II-4 shows the APHIS data (as compiled by HDI) for FY 1982, by type of institution. The numbers for mice and rats are those reported by the institutions that listed those species. Note that the largest users of animals are medical schools and universities, biomedical research institutions and pharmaceutical companies, followed by federal institutions.

Table II-5 shows the APHIS FY 1983 data (as compiled by HDI), for the reporting institutions only.

Table II-6 shows the results for a miscellaneous set of sources, and Table II-7 for a further set of such sources.

TABLE II-1

Institute Of Laboratory Animal Resources Surveys Of Laboratory Animal Use (1960, 1965-1971)

		YEAR							
		1960	1965	1966	1967	1968	1969	1970	1971
Number of Reporting Facilities		561	918	1,442	1,366	1,523	2,258	1,523	1,834
Family	Species								
Rodents	Total	6,208,036	35,410,974	37,442,693	38,309,708	38,880,977	49,748,626	37,247,377	42,126,469
	Mice	4,639,653	24,221,623	27,925,483	27,094,260	26,749,297	35,625,304	25,687,067	30,281,783
	Rats	1,266,046	9,941,497	7,940,449	9,204,735	10,266,682	12,321,871	9,870,628	10,204,069
	Hamsters	131,452	890,716	838,282	1,097,360	808,988	855,819	870,056	938,065
	Guinea Pigs	170,885	346,778	660,282	847,393	987,437	888,602	737,899	646,322
	Gerbils		3,745	66,197	61,460	58,651	39,940	41,874	49,541
	Other Rodents		6,615	12,035	4,000	9,922	17,090	39,853	6,689
Rabbits	Total	187,361	246,958	363,288	639,145	613,641	576,956	494,591	442,614
Carnivores	Total	227,386	132,843	569,255	517,889	593,756	547,884	247,310	274,977
	Cats	45,915	32,910	142,754	136,749	171,322	162,808	56,646	75,935
	Dogs	181,471	92,945	415,159	371,024	409,472	372,229	182,728	192,524
	Ferrets		6,922	6,342	8,116	9,992	6,736	4,664	3,587
	Other Carnivores		66	5,000	2,000	2,970	6,111	3,272	2,931
Ungulates	Total	9,046	55,868	113,590	88,263	91,948	158,715	95,636	275,201
	Swine	4,361	36,844	63,457	52,578	46,624	84,180	42,998	208,346
	Sheep	3,990	11,644	17,182	14,358	22,961	36,075	22,490	22,490
	Horses	695	696	497	228	746	1,322	1,029	1,602
	Goats		2,403	6,450	3,454	3,406	6,338	3,188	3,140
	Cattle		3,370	25,968	17,529	17,991	30,119	25,569	39,156
	Other Ungulates		911	36	116	220	681	362	467

(CONTINUED)

Notes: Data for 1960 obtained from report published in 1964.
Data for 1960 obtained from subset of 561 institutions.

TABLE II-1 ctd.

Institute Of Laboratory Animal Resources Surveys Of Laboratory Animal Use (1960, 1965-1971)

		YEAR							
		1960	1965	1966	1967	1968	1969	1970	1971
Family	Species								
Nonhuman Primates	Total	25,897	63,294	62,783	70,416	85,283	68,002	54,437	56,567
	Old World Primates		55,805	51,749	52,986	50,265	49,148	38,387	38,643
	New World Primates		4,510	11,034	17,430	32,799	17,623	15,284	16,777
	Rhesus Monkeys	9,959							
	Other Nonhuman Primates	15,938	2,979			2,219	1,231	766	1,147
Birds	Total	112,491	902,744	1,812,154	3,654,916	1,278,011	1,909,779	887,263	1,451,020
	Chickens		694,656	1,426,994	3,352,401	1,053,732	1,684,859	636,840	1,272,772
	Pidgeons		50,544	39,081	31,582	66,908	35,296	26,908	22,021
	Ducks		44,202	43,339	22,237	10,896	15,242	13,905	16,998
	Turkeys		40,157	159,950	173,682	58,327	68,814	84,602	29,348
	Quail		54,185	128,390	55,714	64,029	88,614	72,324	80,231
	Other Birds		19,000	14,400	19,300	24,119	16,954	52,684	29,650
Marine Mammals	Total		18		31	33	20	16	62
Fish	Total		11,849	13,500	2,475	277,334	292,184	510,950	843,555
Amphibians	Total		1,892,155	4,293,247	5,000,879	5,480,090	1,712,553	2,039,490	3,366,401
	Frogs and Toads		1,890,955	4,283,097	4,997,870	1,423,208	1,674,650	2,022,755	3,316,827
	Other Amphibians		1,200	10,150	3,009	56,615	37,903	16,735	49,574
	Not Designated					4,000,267			

(CONTINUED)

Notes: Data for 1960 obtained from report published in 1964.
Data for 1960 obtained from subset of 561 institutions.

TABLE II-1 ctd.

Institute Of Laboratory Animal Resources Surveys Of Laboratory Animal Use (1960, 1965-1971)

		YEAR							
		1960	1965	1966	1967	1968	1969	1970	1971
Family	Species								
Reptiles	Total		20,466	48,737	44,886	296,003	100,090	90,697	127,876
	Turtles		766	11,612	7,157	190,318	66,179	69,307	103,721
	Snakes			6,010	32	50,895	1,308	207	888
	Lizards					54,622			
	Not Designated			31,115	37,697	168,411			
	Other Reptiles		19,700				32,603	21,183	23,267
All Animals	Total	6,770,217	38,737,169	44,719,282	48,328,608	47,765,319	55,114,809	41,667,767	48,964,742

Notes: Data for 1960 obtained from report published in 1964.
Data for 1960 obtained from subset of 561 institutions.

TABLE II-2

ILAR National Surveys of Laboratory Animal Facilities and Resources (1967 and 1978)

		Survey Year -- Institution Type					
		1967 All	1967 Federal	1967 Commercial	1967 Non-profit	1978 Non-profit	1978 ALL
Number of Institutions Reporting		1371	197	143	1031	992	1252
Family	Species						
Rodents	Total	30,363,000	9,341,000	11,443,300	9,565,200	8,070,867	18,648,171
	Mice	22,772,300	7,559,200	8,022,000	7,191,100	5,577,299	13,413,813
	Rats	6,131,000	1,220,900	2,976,700	1,933,400	1,959,536	4,358,766
	Hamsters	785,900	374,900	170,300	240,700	259,789	368,934
	Guinea Pigs	613,300	184,500	263,500	165,300	213,822	426,665
	Other Rodents	60,500	1,500	10,800	34,700	60,421	79,993
Rabbits	Total	504,500	60,400	237,900	206,200	299,185	439,986
Carnivores	Total	370,400	38,900	93,900	237,600	167,736	242,961
	Cats	99,300	9,800	30,500	59,000	42,418	54,908
	Dogs	262,000	27,900	59,900	174,200	121,024	183,063
	Other Carnivores	9,100	1,200	3,500	4,400	4,294	4,990
Ungulates	Total	106,200	18,000	40,900	47,300	129,473	144,595
	Swine	53,600	7,800	25,800	20,000	96,618	104,769
	Sheep & Goats	27,700	7,100	4,700	15,900	7,813	12,610
	Cattle & Horses	24,500	3,100	10,300	11,100	24,731	26,897
	Other Ungulates	400		100	300	311	319
Nonhuman Primates	Total	57,700	12,700	23,100	21,900	11,131	30,323
	Old World Primates	43,600	10,800	19,400	13,400	9,014	25,857
	New World Primates	14,100	1,900	3,700	8,500	2,117	4,466
Birds	Total	2,070,500	143,000	1,314,100	613,400	141,543	450,352
All Animals	Total	33,472,300	9,614,000	13,153,200	10,691,600	8,819,935	19,956,388

TABLE II-3

USDA/APHIS (As reported by USDA to Congress)

ANNUAL REPORTS

	YEAR											
	1972 *	1973 *	1974 *	1975 *	1976 #	1977 +#	1978 #	1979	1980	1981	1982	1983
Number of Facilities	732	871	867	985	1,042	1,186	1,233	1,211	975	1,050	1,016	1,005
Species												
Rodents	851,295	863,956	861,205	892,477	989,900	742,274	944,035	876,638	828,216	830,154	797,036	822,071
Hamsters	461,210	454,986	430,766	456,031	503,590	393,533	459,685	419,504	405,826	397,522	337,790	337,023
Guinea Pigs	390,085	408,970	430,439	436,446	486,310	348,741	484,350	457,134	422,390	432,632	459,246	485,048
Rabbits	379,375	447,570	425,585	448,530	527,551	439,003	519,029	539,594	471,297	473,922	453,506	466,810
Carnivores	265,185	261,352	273,463	205,928	293,331	238,741	288,421	280,207	257,265	246,739	211,319	227,886
Cats	79,397	66,195	74,259	51,439	73,333	62,311	71,283	69,103	68,482	58,090	49,923	53,344
Dogs	185,788	195,157	199,204	154,489	219,998	176,430	217,138	211,104	188,783	188,649	161,396	174,542
Nonhuman Primates	42,658	42,298	51,253	36,202	52,083	53,116	64,295	59,359	56,024	57,515	46,388	54,926
Wild Animals	33,450	38,169	81,021	42,523	73,736	46,535	63,893	76,247	49,102	50,111	69,043	108,549
All Animals	1,571,963	1,653,345	1,692,527	1,625,660	1,936,601	1,519,669	1,879,673	1,832,045	1,661,904	1,658,441	1,576,556	1,680,242

* Totals do not include reports from federal agencies
+ Covers the period 1/1/77 - 9/30/77 only
# Totals adjusted from those in annual report to include federal agencies

TABLE II-4

USDA/APHIS
FY 1982
Usage of Species by Institution (As compiled by HDI)

Reporting Institutions Only

		Institution Type									
		Universities & Med Schools	Hospitals Non-University	Biomedical Research	Toxicology Testing Labs	Chemical Companies	Pharmaceutical, Device & Diag	State & Local Gov.	Food, Feed & Misc.	Federal Agencies	TOTAL
Institutions Reporting		410	129	167	79	27	145	19	26	125	1,127
Rats		1,079,208	86,472	343,915	97,237	176,874	558,630	11,299	12,700	359,479	2,725,814
	% row	39.6	3.2	12.6	3.6	6.5	20.5	0.4	0.5	13.2	100
Mice		1,678,300	203,768	1,579,664	431,464	161,659	1,669,629	200,150	6,247	958,863	6,889,744
	% row	24.4	3.0	22.9	6.3	2.3	24.2	2.9	0.1	13.9	100
Dogs		98,983	13,622	22,291	3,457	2,194	37,604	322	3,698	12,696	194,867
	% row	50.8	7.0	11.4	1.8	1.1	19.3	0.2	1.9	6.5	100
Cats		31,555	2,716	7,697	137	115	9,073	87	2,040	3,541	59,961
	% row	57.6	4.5	12.8	0.2	0.2	15.1	0.1	3.4	5.9	100
Guinea pigs		82,198	6,104	25,225	35,145	18,182	272,405	9,044	1,504	48,053	497,860
	% row	16.5	1.2	5.1	7.1	3.7	54.7	1.8	0.3	9.7	100
Hamsters		151,365	5,501	65,146	12,954	3,160	131,227	8,401	23	39,490	417,267
	% row	36.3	1.3	15.6	3.1	0.8	31.4	2.0	0.0	9.5	100
Rabbits		173,716	15,171	63,863	60,785	20,970	177,289	2,102	1,862	31,554	547,312
	% row	31.7	2.8	11.7	11.1	3.8	32.4	0.4	0.3	5.8	100
Primates		23,353	557	13,543	2,577	144	7,709	329	66	6,287	54,565
	% row	42.8	1.0	24.8	4.7	0.3	14.1	0.6	0.1	11.5	100
All Animals		3,321,678	333,911	2,121,344	643,756	383,298	2,863,566	231,734	28,140	1,459,963	11,387,390
	% row	29.2	2.9	18.6	5.7	3.4	25.1	2.0	0.2	12.8	100

TABLE II-5

USDA/APHIS
FY 1983
Usage of Species by Institution (As compiled by HDI)

Reporting Institutions Only

		Institution Type									
		Universities & Med Schools	Hospitals Non-University	Biomedical Research	Toxicology Testing Labs	Chemical Companies	Pharmaceutical, Device & Diag	State & Local Gov	Food, Feed & Misc.	Federal Agencies	TOTAL
Institutions Reporting		102	140	159	86	31	155	18	32	123	1,146
Rats		1,234,864	106,430	406,936	144,162	114,215	778,425	30,378	14,355	439,729	3,269,494
	% row	37.8	3.3	12.4	4.4	3.5	23.8	0.9	0.4	13.4	100
Mice		1,951,466	222,080	1,512,424	495,067	158,752	2,021,157	477,250	3,632	1,071,339	7,913,167
	% row	24.7	2.8	19.1	6.3	2.0	25.5	6.0	0.0	13.5	100
Dogs		90,001	12,605	21,483	5,003	1,591	38,311	436	3,400	9,595	182,425
	% row	49.3	6.9	11.8	2.7	0.9	21.0	0.2	1.9	5.3	100
Cats		32,535	2,265	6,768	172	44	8,624	72	2,092	2,774	55,346
	% row	58.8	4.1	12.2	0.3	0.1	15.6	0.1	3.8	5.0	100
Guinea pigs		64,554	7,195	30,696	28,753	14,722	297,849	10,090	930	66,448	521,237
	% row	12.4	1.4	5.9	5.5	2.8	57.1	1.9	0.2	12.7	100
Hamsters		115,483	5,472	169,272	11,922	612	112,618	3,193	22	35,885	454,479
	% row	25.4	1.2	37.2	2.6	0.1	24.8	0.7	0.0	7.9	100
Rabbits		158,058	15,042	64,626	55,785	22,034	159,276	1,948	2,504	29,779	509,052
	% row	31.0	3.0	12.7	11.0	4.3	31.3	0.4	0.5	5.8	100
Primates		22,201	1,059	13,272	5,809	25	9,376	243	82	7,269	59,336
	% row	37.4	1.8	22.4	9.8	0.0	15.8	0.4	0.1	12.3	100
All Animals		3,669,162	372,148	2,225,477	746,673	311,995	3,425,636	523,610	27,017	1,662,818	12,964,536
	% row	28.3	2.9	17.2	5.8	2.4	26.4	4.0	0.2	12.8	100

TABLE II-6

Miscellaneous Estimates, Projections and Reports

		Source -- Year				
		W B Saunders (Estimate) 1965	W B Saunders (Projection) 1970	DOD Internal Use 1982	NIH Intramural 1983	Rowan (Estimate) 1984
Family	Species					
Rodents	Total	58,440,000	94,480,000	388,143	727,590	62,000,000
	Mice	36,840,000	59,560,000	288,223	504,054	45,000,000
	Rats	15,660,000	25,320,000	70,378	200,468	15,000,000
	Hamsters	3,300,000	5,340,000	11,811	7,941	1,000,000
	Guinea Pigs	2,520,000	4,070,000	17,731	15,127	1,000,000
	Exotic Species	120,000	190,000			
Rabbits	Total	1,560,000	2,520,000	6,098	7,816	750,000
Carnivores	Total			3,783	1,140	350,000
	Cats			813	390	100,000
	Dogs			2,970	750	250,000
Ungulates	Total					200,000
Nonhuman Primates	Total			1,632	4,005	25,000
Birds	Total					5,000,000
Amphibians	Total					3,000,000
	Frogs and Toads					3,000,000
Misc Animals	Total			6,737	931	
All Animals	Total	60,000,000	97,000,000	406,393	741,482	71,325,000

TABLE II-7

Miscellaneous Sources of Data
Annual Usage Rates

Source		
Foster D. Snell	Rats:	40,000,000 produced in 1975, US
	Mice:	35,000,000 produced in 1975, US
	Monkeys:	20,000 imported in 1975, US
Alex Brown & sons (Charles River Breeding Labs, Inc.)	Animals:	22,000,000 worldwide, 1981
Emmons	Frogs:	9,000,000 shipped, 1969
Culley	Frogs:	1,000,000 used, 1981
	Tadpoles:	2,000,000 used, 1981
Nace	Frogs:	9,000,000 shipped, 1971
		4,500,000 shipped, 1981
		4,500,000 shipped, 1984
Various	Fish:	over ½" long 500,000-1,000,000
		to ½" long 2,000,000-3,000,000
James Will	25,000-100,000 avian species for laboratory use	

III. CRITICAL EVALUATION OF SOURCES OF SUMMARIZED DATA

A. Introduction

In order to evaluate the confidence that can be accorded or the reliability of the various data sources, the following parameters were considered:

1. Ability to trace the methodology used in producing the numbers, including the survey technique;
2. Ability to extrapolate to non-reporting institutions, which implies that there is a clear statement as to which institutions reported data, and which did not;
3. Whether data were obtained in some formal manner, or by means of a few interviews, resulting in broad estimates;
4. Ability to determine fraction of animals reported as being actually used in lab experimentation, as contrasted to, for example, animal husbandry.

These parameters were chosen as they have implications for the ability to use the necessarily limited numbers for extrapolation to the entire population of institutions, usually. In general, if the numbers cannot be justified through some rational process, one should not impute too much significance to them. Note that the scores below refer only to the published numbers, and not to their usefulness as a predictive tool.

The creiteria enumerated above were then used to assign a confidence score shown below:

0 = not reliable
1 = reliability questionable
2 = somewhat reliable
3 = fairly reliable
4 = quite reliable
5 = very reliable

The confidence levels were assigned by an admittedly arbitrary process based on the four criteria enumerated above. While a more formal scheme could have been used, the assignment of relative weight to each of the criteria would have been very difficult.

B. Evaluation of Sources

In this section, it is well to point out that the term "use" is interpreted differently by various sources. Some sources will use the term to reflect number of animals acquired, others as actually used in laboratory experiments. This distinction is frequently obscured in the data sources, and only careful reading of the documents and, sometimes, personal inquiry, revealed the true meaning of the numbers.

1. ILAR Surveys

The Institute for Laboratory Animal Resources (ILAR) is a subsidiary of the National Academy of Sciences - National Research Council. It periodically performs surveys of users of lab animals, although it is generally more concerned with facilities and pesonnel than with quantity of animals used. Note that the ILAR data represent the number of animals "acquired by own breeding and from commercial sources," not necessarily the number actually used in experimentation.

a) ILAR (1964) surveyed 561 non-profit, non-federal medical research institutions in the United States in 1960. The animal use results are presented in Table II-1. Not only is the data now 24 years old, but no attempt was made (nor is it possible) to normalize for those institutions not responding.
(CONFIDENCE = 2, somewhat reliable).

b) ILAR (1965-71) Surveys of Laboratory Animal Use consist of tables summarizing the results of questionnaires pertaining to number of animals used for research (see Table II-1). ILAR personnel cannot answer who was surveyed and who responded, thus extrapolation for missing data is impossible. The results, too, are quite old. They should, however, be of some use in assessing trends during the period covered.
(CONFIDENCE = 2, somewhat reliable).

c) The 1968 Survey of Laboratory Animal Facilities and Resources (ILAR, 1970) appears to have been a very thorough (statistically sound) survey including all known users of lab animals. The animal usage results for FY 1967 are in Table II-2. The results shown, however, are only for the 683 federal-grant-eligible organizations which responded because of the survey sponsor's (NIH) interests. It is, however, possible to normalize for missing data based on the reported biomedical research expenditures for these 683 organizations of $920 million in FY 1967.

The results for all respondents, while not mentioned in the report, were compiled and reported for comparison purposes in the 1980 Survey (see Table II-2 for 1967 results).
(CONFIDENCE = 3, fairly reliable).

d) The ILAR (1980) National Survey of Laboratory Animal Facilities and Resources also appears to be a very thorough and statistically solid report, although the data (FY 1978) are now 6 years old. Because it was also funded by NIH, primary attention was given to non-profit federal-grant-eligible biomedical research institutions (see Table II-2). However, data were also received from federal organizations, commercial research labs, and the pharmaceutical industry. 72% of the 2,637 questionnaires were returned; 47% of those were acceptable, thus providing 1,252 respondents (including 992 non-profit federal-grant-eligible, 137 commercial, 25 components of the Department of Defense, 21 units of NIH and 77 components of other federal agencies.) Although we do not know the identities of the respondents, we do know the biomedical research expenditures of the non-profit organizations and their data are reported separately from all respondents, thus we can attempt to extrapolate to the unknown cases based on the known national (meaning "all use in the U.S.") biomedical research expenditures (see Section IV).
(CONFIDENCE = 3, fairly reliable).

2. USDA Animal and Plant Health Inspection Service (APHIS) is responsible for enforcing the Animal Welfare Act. This includes compiling and reporting the results of the Annual Reports of Research Facilities which are required by law to be reported by each facility. These have been collected since 1972 (see Table II-3 for 1972-83; and Tables II-4 and II-5 for 1982 and 1983 results).

Animals covered by the Act include live dogs, cats, guinea pigs, hamsters, rabbits, non-human primates, and any other warm-blooded animal used in research and either (1) purchased or transported in commerce or (2) used in research supported by the federal government. Elementary and secondary schools are exempt. Not included would be dead frogs used in biology classes or animals killed prior to usage. Rats and mice are also not covered, thus report of their use is voluntary (although they have been listed on the form since 1982). A facility using only exempt species need not file a report. This means that a facility that only uses rats or mice need not file a report. Inquiries of workers in the field indicate, however, that the number of rats and mice not reported in this fashion is very small, probably less than 2% of the total. While legally voluntary, many respondents filled in numbers for rats and mice anyway; many, we have found, did not even realize that reporting these species was voluntary.

The 1982 and 1983 data were analyzed on a case-by-case basis. Copies of the original report forms were obtained from the Animal Care Staff at USDA; they were then sorted by institution type, checked, coded and entered into a computer data base. They are listed in Appendix C. The results are shown in Tables II-4 and II-5, by institution type. It should be noted that, in many cases, this sorting process was arbitrary. For example, the Kendall Research Labs form could have been placed in either (1) for-profit research agencies, (2) pharmaceutical manufacturing or (3) medical device manufacturers. Likewise CIBA-GEIGY produces both pharmaceuticals and chemicals, thus either categorization would have been permissible. Also it was often difficult to distinguish non-profit from for-profit research. Finally, the numbers of cases reported under each institution type do not necessarily reflect the actual number of institutions. This is because some institutions (especially higher education) require each department or facility to fill out its own form (i.e., Psychology, Biology, Pharmacology, Medical School) while some fill out one form encompassing all departments. Thus, some medical schools numbers are reported under the institution with which they are affiliated.

We would like to caution against comparing the APHIS data presented here with the Annual USDA Summary Reports. Those reports will invariably contain lower numbers for all species, due to the fact that those data sheets that are received by USDA after a cut-off date (usually December 31), are not included in their reports, neither for the current nor the next fiscal year. We estimate that between 10 and 20% of the reports thus are not used to compile the report to Congress in a given year. This limitation does not apply to our results, since we used a given year's data, no matter when it was received. The assumption had to be made, however, that we had received copies of all, or substantially all, of the data sheets. Time did not permit to verify which institutions did not report. That would have been possible, however.

It is felt that, for the six required species listed on the form, the numbers reported provide a very close approximation to the actual numbers used.
(CONFIDENCE = 5, very reliable - for mandated species).

For exempt species (primarily rats and mice), it is felt that anyone who takes the time and effort to report data will provide valid data for the exempt species as well. Based on this assumption we expect to be able to extrapolate the number for unreported rats and mice based on those who did report (see Section IV).
(CONFIDENCE = 4, very reliable for reported data - rats and mice).

For exempt species, it has been pointed out (Abood, 1984; Bier, 1984; McArdle, 1984) that a certain number of animals go unreported because they are purchased directly by the user and not reported to the central facility. This could not be confirmed, however.

3. Saunders & Company (1966) performed a survey of the laboratory animal market in 1965 and projected to 1970. The results are shown in Table II-6. The company no longer exists, and since the survey methodology is unclear, no evaluation is possible.
(CONFIDENCE = 0, unreliable).

4. A study performed by Foster D. Snell, Inc. for the Manufacturing Chemists Association (1975) estimated that 35 million mice and 40 million rats were produced domestically (per year) in the U.S. in 1975, and that 20,000 monkeys were imported from India.

   The writer(s) of the report could not be located and the methodology is unclear, thus it is impossible to validate the methodology. It appears that the data are based on interviews with personnel at two animal breeding facilities (Charles River, White Eagle Farms) and perhaps a few other people in industry, academia and government. Thus it is difficult to give any credibility to the data.
(CONFIDENCE = 0, unreliable).

5. Alex Brown & Sons (1981) report on Charles River Breeding Labs, Inc. states that Charles River produces 22 million animals annually worldwide, specializing in mice, rats, guinea pigs, hamsters and primates. It does not give any breakdown by species, nor do any of the other analyses of Charles River.
(CONFIDENCE = 2, somewhat reliable). Because it is merely a guess based on a few interviews, its value must be questioned.

   Charles River is estimated to have 20% (in dollars) of the U.S. laboratory animal market. A current employee at Bausch & Lomb, which purchased Charles River in 1983, feels that this figure (20%) is "... both outdated and low ..." but declined further comment (Anon, 1984).

   Using various other parameters, one can estimate the numbers of each species produced by Charles River (see Section IV) and then extrapolate, based on market share, to the entire U.S. One problem in this extrapolation is that the "waste factor" inherent in animal breeding is ignored: a significant proportion of the animals bred for research die, or do not meet protocol specifications (age, sex, weight, general health) and must be discarded. This factor must be borne in mind when comparing "production" with "use."

6. Amphibians

a) Amphibian use is not required to be reported on the USDA/APHIS annual reports. The most recent of the surveys to assess amphibian use were the ILAR Surveys of 1965-71 (Table II-1), indicating the use of 3.37 million amphibians in 1971. As mentioned, however, it is not known how to normalize for those institutions which did not report, thus the usefulness of these data are questionable. (CONFIDENCE = 1, of questionable reliability).

b) Emmons (1980). Marvin Emmons, formerly general manager of one of the major suppliers of amphibians, estimates that approximately 9 million frogs were shipped by suppliers in 1969 for educational and teaching purposes.
(CONFIDENCE = 1, of questionable reliability). Because it is a global estimate, we question the significance.

c) Bud Culley (1984), an amphibian researcher at Louisiana State University performed a survey of the use of bullfrogs in 1981. He estimated that roughly 150,000 bullfrogs and 200,000 tadpoles could have been used in 1981 (a decrease since 1971, he found). Assuming bullfrogs comprise roughly 10% of the total frequency of use, he estimated that about 1 million frogs and 2 million tadpoles were used yearly in the U.S. for teaching and research.
(CONFIDENCE = 1, of questionable reliability). Because of the very general assumptions made here, the value of this estimate is questioned.

d) George Nace (1984b), estimated that about 9 million frogs were shipped by suppliers in 1971, but that this dropped to roughly 4½ million in 1981 and has stabilized at 4½ million in 1984 with 90% of

the usage educational and 10% research. 100% of the frogs used are collected in the wild by "frog farmers."
(CONFIDENCE = 3, fairly reliable). There is fairly good detail for the basis of the estimates, but it is difficult to know the actual numbers due to the large number of users.

7. Fish Usage

Data on usage of fish for laboratory purposes were particularly difficult to obtain with any degree of reliability. The best numbers were not much better than estimates from users in the field.

Estimates were obtained from commercial and institutional (including government) users. For fish over ½ inch long, the yearly use appears to range between 500,000 and one million. For fish smaller than ½ inch, the best estimate is that 2 to 3 million are used yearly. Most of these fish are used for toxicological studies.

These numbers apply only to laboratory use. They do not include the millions of fish that are used in the wild in propagation, contamination, feeding, and other ecological studies, such as those associated with projected dams. It is essentially impossible to estimate the number of fish used for these purposes. It would, however, be stretching the term "laboratory" pretty far to consider these animals to be used in such a setting. This is particularly so since in these settings, the fish are usually not bred for the purpose for which they are used, but grow in the wild.
(CONFIDENCE = 3, fairly reliable). While the numbers are fairly consistent from source to source, they are only global estimates.

8. Birds

The APHIS data sheets by institution report 31,802 birds for FY 1982, and 37,221 for FY 1983. The table below shows how these are allocated for the more important species:

	1982	1983
Fowl, domestic	13,200	11,494
Chicken	8,334	8,096
Quail, coturnix	5,303	2,125
Quail, unspecified	1,059	1,786
Pigeons	1,796	3,699
Ring dove	1,133	100
Bird, unspecified	3,085	2,481

Of the above, the University of Maryland used 17,915 birds in FY 1982, and 12,305 in FY 1983. Since this one institution used such a large fraction of the reported total, inquiries were made as to who the other possibly nonreporting large users might be. Naturally enough they turned out to be the poultry research institutions, mostly land-grant universities in the East and South. The largest of these, in terms of poultry research, is North Carolina State. This institution did not report its birds on the APHIS form, but we were able to obtain the following approximations from the University:

	Poultry Science	Veterinary Schools
Domestic fowl	30,000	1,000
Turkeys	6,000	-0-
Quail	5,000	100

Checking the APHIS data sheets for other land-grant institutions determined that most of these had reported their bird usage.

Discussions with researchers at several institutions brought out the fact that only 80-85% of the poultry science usage is for laboratory use, the remainder mostly for feeding, management, and breeding studies.

When all these uncertainties and data are brought together, the estimate made by Dr. James Will, of the Animal Resource Center at the University of Wisconsin of 25,000-100,000 avian species used for laboratory experimentation is probably as well as one can do.

While there is good detail for many institutions, there is uncertainty about non-reporting and uncertainty pertaining to the fraction of fowl used in actual experimentation.
(CONFIDENCE = 2, somewhat reliable).

The evaluations of the data sources are summarized in Table III-1.

TABLE III-1

Reliability of Various Data Sources for numbers of Animals Used

Source	Date	Confidence Level	Strengths	Limitations
1. ILAR	1964	2		Data 24 years old. Cannot normalize for non-reporting institutions. Limited to non-profit, non-federal medical research firms.
2. ILAR Surveys of Laboratory Animal Use	1965-71	2	Of some use in establishing trends for that period	Old data. Cannot extrapolate to mising data.
3. 1968 ILAR Survey of Lab Animal Resources and Facilities	1967	3	Statistically sound survey. Possibility of extrapolating to other institutions.	Limited to 683 federal-grant-eligible institutions.
4. 1980 ILAR Survey of Lab Animal Facilities and Resources	1978	3	Thorough and statistically solid. Extrapolation to non-reporting institutions possible.	Primary attention given to non-profit federal-grant-eligible institutions. Not required by law to be filled out.
5a. USDA APHIS mandated species	1982-83	4	Required by law. Data available by institution thus extrapolation to non-reporters possible.	
5b. mandated species	1972-81	3	Required by law. Data by institution available, but not utilized	10-20% of institutions not included in report to Congress. Totals not consistent (some years include federal agencies, some years do not).
5c. exempt species	1982-83	4	Data by institution available. Rats and mice were on the form. Anyone who reported probably provided an accurate number. Many did not realize that these were voluntary since they were listed on form. Extrapolation possible.	Not required by law.
5d. exempt species	1972-81	NA		Not required. Not on forms.

TABLE III-1 (continued)

Reliability of Various Data Sources for numbers of Animals Used

Source	Date	Confidence Level	Strengths	Limitations
6. Saunders & Co. Survey	1965	0		Company defunct, survey methodology unclear; no evaluation possible.
7. Foster D. Snell for MCA	1975	0		Data appears to be based on interviews with 2 breeders. Methodology unclear. Personnel no longer available.
8. Alex Brown & Sons	1981	2	At the time, it was thought to represent best estimate for lab animals in U.S. market.	Data based on a few interviews, and mostly broad estimates.
9. Emmons	1969	1-2		Global estimate
10. Culley	1981	1		Many assumptions.
11. Nace	1971-84	3	Fair detail for basis of estimates	Difficult to know actual numbers due to large number of users.
12. Various (fish usage)	1983	3	Data initially consistent	Global estimates only.
13. Various (bird usage)	1983	2	Good detail by institutions	Uncertainty about non-reporting institutions, and fraction of fowl used for lab experimentation.

IV. ESTIMATION OF ANNUAL LABORATORY ANIMAL USE

A. Estimation Using Indirect Means

1. Based on NIH Funding

NIH funds 37% of all national biomedical research expenditures (NIH, 1983).

In FY 1983, NIH awarded $582,571,000 in direct costs to 5,011 extramural projects utilizing rats (and other species, including possibly mice). (Bohrer, 1984). NIH currently has no method of breaking this out by species (without overlap). If we assume, for a moment, that all of the expenditures went to projects which used only rats, we could extrapolate to an upper limit for rats purchasable using NIH funds.

12% (on the average) of the direct cost of NIH-sponsored research funds go toward purchase of supplies, glassware, chemicals, research animals and items listed as expendable (Willett, 1983). If one-half of all supply funds went toward the purchase of animals (probably less) then $34,954,260 would be available for the purchase of rats. If we assume the average cost of a rat to be $4.00 (see Appendix A for methodology), then 8.7 million rats could be purchased using NIH funds.

In FY 1983, NIH awarded $531,519,000 from direct costs to 4,080 projects utilizing mice. If we again assume for a moment that all of this expenditure went to projects using only mice then we may extrapolate to an upper limit for mice. If one-half of all supply funds were used to purchase animals, then $31,891,140 would be available for the purchase of mice. At an average of $2.00 per mouse (see Appendix A), 16 million mice could be purchased with NIH funding.

Since NIH provides 37% of national health R&D funding, if we assume that NIH also supports the use of 37% of the lab animals used in the U.S., then we can estimate the potential number of rats and mice which could be purchased in the U.S. as:

RATS = 8.7 million x 100/37 = 23.6 million

MICE = 16 million x 100/37 = 43.1 million

Because there are so many assumptions used for this method we have given it a lowered confidence.
(CONFIDENCE = 1, reliability questionable).

2. Based on National Cancer Institute (NCI) Usage

NCI, the cancer research arm of the National Institutes of Health, supported the use of about 5 million rodents (98%, or 4.9 million, of which were mice) in FY 1983 (New, 1984). In fact, only about 3 million animals (2.94 million mice) were actually utilized directly in research experiments. The remaining 2 million were used primarily as breeding stock in production of those to be issued; also included are throw-aways (those that don't meet protocol requirements) and deaths.

NCI's budget of $983,576,000 equalled 24.6% of NIH's total 1983 budget. If we make the assumption that NCI therefore supports the use of ¼ as many mice as does NIH, then we can extrapolate NIH support of mice as:

NIH-MICE = NCI-MICE x 100/24.6 = 4.9 million x 4.07 = 19.9 million

This agrees with the estimates obtained in Section IV.A.1 for mice. Continuing with the assumption that NIH's use of mice equals 37% of use nationwide, we calculate national use of mice as:

TOTAL = NIH x 100/37 = 19.9 x 2.7 = 53.8 million mice "used" per year

Bearing in mind, however, that only 60% of the 4.9 million NCI mice were actually "used in research," this would indicate that 53.8 x .60 = 32.3 million mice could be used in research each year in the U.S.

EVALUATION: (1) Because NCI uses such an extraordinarily large proportion (98%) of mice compared to all species (75% is more typical),

this estimate would probably be better applied as the estimate of rats and mice together. (2) It is also quite possible that NCI's usage of animals as a function of research dollars is considerably higher than other NIH research programs. Thus, NCI may actually support the use of more than ½ of all animals used in NIH-funded research. (3) There are many other assumptions made here. For these reasons, it is recommended that this figure be used only as a potential maximum for the numbers of rats and mice used in U.S. research.
(CONFIDENCE = 1, reliability questionable).

3. Based on NIH Total Usage as a Function of Intramural Use

In FY 1983, NIH funded 525 projects utilizing mice intramurally (within agency labs, by NIH personnel) (Bohrer, 1984). This represented 11.4% of the 4,605 (active) projects referencing mice by NIH's CRISP computer system. If we assume that 11.4% of all mice supported by NIH funding are used intramurally then total use may be estimated as:

TOTAL-NIH-MICE = INTRAMURAL x 100/11.4 = 504,054 x 8.8 =
4,421,526

Using the argument that NIH funds 37% of national biomedical research, thus 37% of animal use, we can estimate the use of mice nationwide as:

MICE = NIH x 100/37 = 4.42 million x 2.7 = 11.9 million mice/year.

Similarly for rats, 9.5% (527) of the total (5,538) active NIH projects used 196,458 rats intramurally (Bohrer, 1984). Thus usage may be estimated as:

TOTAL-NIH = INTRAMURAL x 100/9.5 = 196,458 x 10.5 =
2.1 million rats

Again, national use may be estimated as:

TOTAL RATS = NIH x 100/37 = 2.1 million x 2.7 = 5.6 million rats/year.

(CONFIDENCE = 1, reliability questionable). Again, because so many assumptions are made, this methodology is given lowered confidence.

4. As a function of estimate of animals bred (especially rats and mice)

a) Analysis of Charles River Breeding Laboratories, Inc. sales.

Charles River Breeding Labs, now a subsidiary of Bausch & Lomb, Inc., is the largest breeder of lab animals in the U.S. Charles River sales in 1982 were $40.9 million, of which $29.5 million were domestic (Charles River, 1982). 80% of total sales in 1981 were from the sales of rats and mice (Brown & Sons, 1981). Assuming that 80% of domestic sales also were due to rats and mice, then $23.6 million would be attributed to domestic sales of rats and mice. Generally, mice and rats are used in research in the proportion of 3:1 (ILAR, 1980; USDA, 1982). However, because rats cost roughly twice as much as mice (see Appendix A), rats theoretically contribute 2/5 (or $9.4 million) and mice 3/5 (or $14.16 million) to their combined sales.

If an average mouse sells for $2.00 (see Appendix A), then Charles River could theoretically have sold approximately 7.1 million mice in 1982. Alex Brown & Sons (1981) estimated Charles River to have 20% of the small laboratory animal (primarily rat and mouse) market share in 1981. A source at Bausch & Lomb (the current owner of Charles River), however, while not willing to quote a figure, feels that 20% is "... both outdated and conservative ... (low)." (Anon, 1984). If we assume 25%, then 28.3 million mice could have been sold by commercial breeders in 1981. The ILAR (1980) study found that 65% of mice acquired for research were obtained from commercial sources, and 35% were bred in-house by the researchers. Thus, total national usage of mice may be estimated to equal 28.3 x 100/65 = 43.6 million mice in 1982.

Similarily, if rats contribued $9.4 million to 1982 sales, and a rat on the average costs approximately $4.00, then 2.36 million rats could theoretically have been sold by Charles River. Assuming a 25% market share permits an estimate of 9.4 million rats sold commercially. The

ILAR (1980) survey found that 81% of rats were purchased commercially while 19% were bred in-house. Thus total national use of rats in 1982 can be estimated to be:

TOTAL-RAT-U.S. = COMMERCIAL x 100/81 = 9.4 million x 1.2
= 11.7 million rats

EVALUATION: Because so many assumptions have been made, the results of this methodology can be given very little or no confidence. (CONFIDENCE = 1, reliability questionable).

b) Animal use estimate as function of estimates of animals bred (based on Charles River Estimates).

Henry Foster (1983) then its President, stated that Charles River "produced ... in excess of 22 million animals annually." Since mice and rats together accounted for 80% of Charles River sales in 1981 (Brown & Sons, 1981), it is probably safe to assume that these species accounted for 85-90% of the numbers of animals sold. Assuming 90%, then Charles River sold nearly 19.8 million mice and rats. 70% of these sales were domestic, so assuming that 70% of rats and mice sold were used in the U.S., yields an estimate of 13.9 million rats and mice sold in U.S. From ILAR (1980) and USDA (1982) one sees that mice and rats are used in the proportion of 3:1. With this assumption, 10.4 million mice and 3.5 million rats were sold yearly by Charles River in the U.S. If Charles River has 25% market share (see Section IV(A)4a), then 41.7 million mice and 13.9 million rats are sold yearly by commercial breeders.

65% of mice used in U.S. research are acquired commercially, thus 41.7 x 100/65 = 64 million/yr. are used in the U.S. according to this method.

81% of rats used in U.S. research are acquired commercially, thus 13.9 x 100/81 = 17.2 million rats are used yearly in the U.S., according to this methodology.
(CONFIDENCE = 1, reliability questionable).

B. Estimation Using Corrected Data from ILAR 1978

Using the results of the 1978 Survey (ILAR, 1980, Table II-2) one is able to approximate animal use for all users with techniques to fill in the missing data of non-respondents based on the following methods:

1. Method a.

The NIH-grant-eligible non-profit biomedical research organizations responding to the survey reported biomedical research expenditures of $2.2 billion for 1978. Total national biomedical research expenditures are estimated at 6.27 billion for 1978 (NIH, 1983). If we assume that (1) animal use (in numbers) is proportional to the dollar amount spent on research utilizing them, and (2) that the usage rate of animals by all institutions is proportional to that of non-profit institutions, then national usage may be estimated as:

TOTAL = (NON-PROFIT ILAR 1980) x 6.27/2.2

Using this equation animal use estimates for 1978 are made starting from Table II-2. The results are shown in Table IV-1. (CONFIDENCE = 2.75, fairly reliable). Because the two assumptions are not readily justifiable, the confidence in this method must be lowered.

2. Method b.

Estimated response rate and funding share of non-profit institutions:

i) NIH supported 41% of all "health" (medical and health-related) R&D in the U.S. in 1978 (NIH, 1983) and 91% of NIH awards go to private, non-profit institutions; therefore NIH-grant-eligible biomedical research organizations use 37% of all biomedical research funds in the U.S. Thus it can be assumed that NIH-grant-eligible biomedical research organizations use 37% of all laboratory animals.

ii) Non-profit agencies account for $3.1 billion in biomedical research expenditures in 1978 (NIH, 1983). Thus the non-profit survey

TABLE IV-1

Estimates of Animals Acquired, 1978
based on ILAR data

	Method		
	(a)	(b)	(c)
Mice	16,000,000	21,600,000	19,200,000
Rats	5,600,000	7,700,000	6,200,000
Other rodents	1,500,000	2,060,000	1,251,000
Rabbits	850,000	1,154,000	629,000
Carnivores	480,000	650,000	347,000
Ungulates	370,000	500,000	207,000
Birds	400,000	550,000	640,000
Non-human primates	32,000	43,000	43,400
TOTALS	25,232,000	34,257,000	28,517,400
CONFIDENCE in estimates	(3)	(3)	(1)

response accounts for 70% (2.2/3.1) of that total. Assume, then, that 70% of the animals used by non-profit agencies are reported.

Using these two assumptions then, animal use may be estimated as:

TOTAL = (NON-PROFIT ILAR 80) x 100/37 x 10/7
= 3.86 x (NON-PROFIT - ILAR 80)

Using this equation and starting from Table II-2, estimates of total national animal use in 1978 are shown in Table IV-1. (CONFIDENCE = 3, fairly reliable). Because these two assumptions cannot be readily justified, this method must be given a lowered confidence.

3. Method c.

Estimated response rate of all respondents:

Assuming that the same response rate (70%) holds for all respondents (as did for non-profit), animal use may be estimated (starting from Table II- 2) as follows:

TOTAL = (REPORTED - all respondents) x 10/7

The results using this methodology are shown in Table IV-1.

Since we cannot verify the response rate of other (not non-profit) respondents, we must assign lower confidence to this methodology. (CONFIDENCE = 1, reliability questionable).

C. Estimation Using Corrected Data for APHIS Summary Reports for FY 1982 and FY 1983

1. Rats and Mice

For about two-thirds of the institutions, respondents volunteered the number of rats and mice used on the 1982 and 1983 forms. It is felt that those who did so had no reason to misrepresent their data; thus it is assumed to be of very good quality.

For each of the years 1982 and 1983, separately, regression equations based on those institutions reporting the specific species were developed. These equations were then used to estimate the numbers of rats and mice for those institutions not reporting these species. The data for these regressions were the APHIS reports, by institution. These reports were obtained directly from USDA. The data are listed, individually, in Appendix C.

On receipt of the reports, they were sorted and coded by institution type, duplicates removed, and keystroked. Thereafter, a data base was created for each year, and standard data processing techniques were used to verify the data.

The independent variables that were considered included the number of animals for which reporting is mandated, i.e., cats, dogs, non-human primates, guinea pigs, hamsters and rabbits. In addition, higher order functions of these variables were also allowed. Also, indicator (0, 1) variables were permitted for the various institution types.

Distributional tests on the continuous variables were made. These indicated that logarithmic transformations were necessary, mostly due to the wide range of numbers.

The resulting regression equations are shown in Appendix B. The estimates obtained using these regression equations are shown in Table IV-2. Also shown in Table IV-2 are the estimates obtained by simply applying the mean value for reporting institutions to the non-reporters.

Note that there are very considerable differences in rat and mouse use estimation depending upon whether the basis of the estimation is number of animals acquired or used, as was pointed out in Section III-B. As will be discussed in Section V-D, many of the major differences in Table IV-2

TABLE IV-2

Summary of extrapolation methodology results for rats and mice

			Mice x 10^6		Rats x 10^6		Confidence Level
			1982	1983	1982	1983	
Basis of Estimation of use method (Section IV)							
	C.1	USDA/APHIS					
		Regression	8.5	8.5	3.4	3.7	4
		Average	10.2	11.2	4.1	4.6	4
*	A.1	NIH Funding		43.1		23.6	1
	A.2	NCI Usage		32.3			1
	A.3	NIH Usage (Intramural)		11.9		5.6	1
*	A.4	Animals bred	44-64		12-17		1
*		ILAR		1978		1978	
	B.1	Non-profit funding share		16.0		5.6	3
	B.2	Non-profit response rate		21.6		7.7	3
	B.3	All respondents		19.2		6.2	1

* Basis: animals acquired

can be explained by this fundamental difference in the basis of estimation.

Dr. Albert E. New, Director of Laboratory Animal Science at the National Cancer Institute stated that, at NCI, roughly 40% of the laboratory animals bred will never be used laboratory experimentation. This would bring the estimates based on method A.4 pretty much in agreement with the APHIS estimates for rats, but not for mice. It must be borne in mind, however, that the number of animals bred is based on several assumptions: that the average price per animal is in fact realistic, and that the market share of Charles River Labs is of the magnitude indicated in the Alex Brown study. Similar limitations obtain for methods A.1-A.3 since these are all based on dollars, not actual animals counted to have been used.

As to the estimates based on the ILAR reports, the major problem is that one does not know who the respondents were, and thus one has no handle on the possible bias in the data. It would be, for example, quite possible that only the largest users of animals responded, and simple extrapolation to the remainder would obviously overstate the number of animals used. The same consideration would of course also obtain in the opposite direction.

The APHIS data, on the other hand, reflect actual numbers of animals used, and involve no assumptions about costs. The only assumption made for extrapolation to the institutions that did not report rats and/or mice is that on an institutional basis, their use of rats and mice could be estimated from their use of non-exempt species. We believe, from having checked oral reports against APHIS data sheets, that the data reported on the data sheets are accurate, within the internal reporting procedures of each institution.

Thus, we would put our faith in the results obtained for the APHIS data sheets. We believe that these results can be believed within -10%, and +25%. They could be lower if the regression equation overestimated the contribution of the non-reporting institutions. They could be higher for

that reason, but also due to a possible tendency to underreport, though we do not think that this is an important factor.
(CONFIDENCE = 4). The estimates obtained using these methods are felt to be quite reliable.

2. Mandated Species

After compiling the data from the Annual Reports of the individual institutions as in the preceding section, the results were tabulated by species and by institution type. These results are presented in Table V-1, including the percentage change from 1982 to 1983. Bearing in mind the problems mentioned in Section III(B)2 regarding the USDA/APHIS data as reported to Congress, the corrected data as presented here (Table V-1) represent a more accurate picture ot true animal use. There may still be minor problems of underreporting and missing data sheets but we do not think that these amount to more than 20%.
(CONFIDENCE = 5, very reliable).

Thus for 1982 and 1983, regardless of institution type, the following numbers of these species have been used:

	1982	1983
Dogs	194,867	182,425
Cats	59,961	55,346
Guinea pigs	497,860	521,237
Hamsters	417,267	454,479
Rabbits	547,312	509,052
Primates, non-human	54,565	59,336
Total	1,771,832	1,781,875

3. Other Species

a) Fish. As stated in Section III.B.7 these numbers are very soft, and could be overestimated by 50%, and underestimated by 100%. Nevertheless, for what they are worth, they are as follows:

TABLE V-1

USDA/APHIS DATA

Changes 1982-1983

Reporting Institutions Only

		Institution Type									
		Universities & Med Schools	Hospitals Non-University	Biomedical Research	Toxicology Testing Labs	Chemical Companies	Pharmaceutical, Device & Diag	State & Local Gov.	Food, Feed & Misc.	Federal Agencies	TOTAL
Species	Year										
Rats	1982	1,079,208	86,472	343,915	97,237	176,874	558,630	11,299	12,700	359,479	2,725,814
	1983	1,234,864	106,430	406,936	144,162	114,215	778,425	30,378	14,355	439,729	3,269,494
	% change	14	23	18	48	-35	39	168	13	22	19
Mice	1982	1,678,300	203,768	1,579,664	431,464	161,659	1,669,629	200,150	6,247	958,863	6,889,744
	1983	1,951,466	222,080	1,512,424	495,067	158,752	2,021,157	477,250	3,632	1,071,339	7,913,167
	% change	16	8	-4	14	-1	21	138	-41	11	14
Dogs	1982	98,983	13,622	22,291	3,457	2,194	37,604	322	3,698	12,696	194,867
	1983	90,001	12,605	21,483	5,003	1,591	38,311	436	3,400	9,595	182,425
	% change	-9	-7	-3	44	-27	1	35	-8	-24	-6
Cats	1982	34,555	2,716	7,697	137	115	9,073	87	2,040	3,541	59,961
	1983	32,535	2,265	6,768	172	44	8,624	72	2,092	2,774	55,346
	% change	-5	-16	-12	25	-61	-4	-17	2	-21	-7
Guinea pigs	1982	82,198	6,104	25,225	35,145	18,182	272,405	9,044	1,504	48,053	497,860
	1983	64,554	7,195	30,696	28,753	14,722	297,849	10,090	930	66,448	521,237
	% change	-21	17	21	-18	-19	9	11	-38	38	4

(CONTINUED)

TABLE V-1 ctd.

USDA/APHIS DATA

Changes 1982-1983

Reporting Institutions Only

		Institution Type									
		Universities & Med Schools	Hospitals Non-University	Biomedical Research	Toxicology Testing Labs	Chemical Companies	Pharmaceutical, Device & Diag	State & Local Gov.	Food, Feed & Misc.	Federal Agencies	TOTAL
Species	Year										
Hamsters	1982	151,365	5,501	65,146	12,954	3,160	131,227	8,401	23	39,490	417,267
	1983	115,483	5,472	169,272	11,922	612	112,618	3,193	22	35,885	454,479
	% change	-23	-.5	159	-7	-80	-14	-61	-4	-9	9
Rabbits	1982	173,716	15,171	63,863	60,785	20,970	177,289	2,102	1,862	31,554	547,312
	1983	158,058	15,042	64,626	55,785	22,034	159,276	1,948	2,504	29,779	509,052
	% change	-9	-.8	1	-8	5	-10	-7	34	-5	-6
Primates	1982	23,353	557	13,543	2,577	144	7,709	329	66	6,287	54,565
	1983	22,201	1,059	13,272	5,809	25	9,376	243	82	7,269	59,336
	% change	-4	90	-2	125	-82	21	-26	24	15	8
Institutions Reporting	1982	410	129	167	79	27	145	19	26	125	1,127
	1983	402	140	159	86	31	155	18	32	123	1,146
	% change	-2	8	-4	8	14	6	-5	23	-2	2

Fish under ½ inch in length	2-3 million/year
Fish ½ inch or longer	500,000 - 1 million/year

(CONFIDENCE = 3, fairly reliable).

b) Birds

As stated in Section III.B.8, the best that can be done is to estimate the use of birds for laboratory experimentation at 25,000-100,000 animals per year.
(CONFIDENCE = 2, somewhat reliable).

V. ANALYSIS OF ESTIMATIONS, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDY.

A. General Profile

From the data presented in previous sections, the following findings can be made: the largest users of laboratory animals are, in decreasing order of use, FY 1983:

	Cumulative %, FY 1983
Universities and medical schools	28.3
Pharmaceutical and related companies	54.7
Biomedical research institutions	71.9
Federal agencies	84.7
Toxicology testing laboratories	90.5

The above are based on the reporting institutions only (Table II-5), but the numbers would not change significantly, it is believed, if the non-reporting institutions were included. Only mice and rats would be affected, at any rate.

By far the largest proportion of animals is represented by mice and rats. For the reporting institutions, mice represent 60.8% of all animals used, and rats

25.1%. The use of rats and mice is not distributed in the same fashion as total animal usage, as can be seen by the following table:

	Cumulative %, FY 1983	
	Mice	Rats
Universities and medical schools	24.7	37.8
Pharmaceutical and related companies	50.2	61.6
Biomedical research institutions	69.3	74.0
Federal agencies	82.8	87.4
Toxicology testing laboratories	89.1	91.8

Again, it is not believed that the inclusion of the non-reporting institutions for rats and mice would make a material difference in these distributions.

As to the mandated species, half or more of cats and dogs are used by universities and medical schools. Guinea pigs are used mostly by the pharmaceutical industry, and hamsters in biomedical research, and to a lesser extent universities, medical schools and the pharmaceutical firms. 62.3% of the use of rabbits is accounted for by universities, medical schools and the pharmaceutical industry, as are 75.6% of the primates.

B. Number of Reporting Institutions

The number of reporting institutions differ considerably depending upon the source of data. The 1960-1971 ILAR surveys used between 651 and 2,258 reporting facilities, whereas the 1978 survey had 1,252 institutions reporting. The differences are accounted by, in the early years, only a small subset being surveyed, and in the later years, too loose a definition of institutions.

The number of institutions reporting to APHIS hovered around 1,000, or slightly more, since 1972. The numbers reported in the APHIS Annual Summary Reports, however, are low by 10-20% since those reports that are received by APHIS after December 31 of a given year are not tabulated. The numbers of institutions for FY 1982 and FY 1983 shown in Tables II-4 and II-5, however, were tabulated from the actual summary data sheets provided to

APHIS by the institutions. Thus these total 1,127 and 1,146 for 1982 and 1983 respectively. Even these numbers are probably low, as not all institutions report. One should bear in mind that the total number of institutions registered by APHIS in 1982 and 1983 were 1,113 and 1,166, respectively (excluding federal agencies, which are not required to register). Some of these institutions may not report because they have not used any animals that year, or they have only used exempt species.

C. Changes of Significance within ILAR and APHIS Surveys

It is difficult to point out significant changes within either the ILAR or APHIS surveys, since for ILAR, the methodology was variable over time, and could not be verified adequately. Insofar as APHIS is concerned, other than the changes in number of reporting institutions at various years, mostly accounted for by the inclusion or non-inclusion of the federal institutions and a short fiscal year, no significant changes could be identified.

D. Comparison of Data from ILAR Surveys and APHIS Reports

The ILAR and APHIS surveys are so different in their organization and methodology that it is not meaningful to compare data from these two sources, even in years in which data from both are available.

E. Trends and Projections of Future Laboratory Animal Use

Table V-1 presents detailed data by institution type for 1982 and 1983 for the mandated species. Also included in that Table are data for rats and mice.

Before proceeding to an interpretation of the data, one must point out that for rats and mice, the trends one could obtain from this table are clouded by the fact that there was more reporting by institutions of rats and mice in 1983 than in 1982. Sufficient time was not available to analyze this effect by institution type, since that would be the only meaningful analysis. So, while it would appear from Table V-1 that the usage of mice and rats increased in 1983 over 1982, this is in fact not so, as can be seen from Table IV-2 which shows the results over all institutions for those two years, though a small increase in the number of rats appears to be present on the basis of the regression estimate. However, since the same pattern of increase by institutional group reporting can be seen from Table V-1, we do not think that there has been any material change between the two years, neither increase nor decrease.

Another comment regarding Table V-1 is that the number of reporting institutions included institutions that reported any number for any species, whether these included rats or mice or not. Again, it would be possible to show separate tables for each of the species with the appropriate numbers of institutions for each table, from which one could calculate average number of animals per institution type for each species, but again, the available resources did not permit that level of detail.

Then examining Table V-1, it is evident that very few changes of any significant magnitude occurred as a function of institution type, with the exception of the number of hamsters used by biomedical research institutions in 1983, in which the number more than doubled over the 1982 usage. This is against the trend of the other hamster-using institutions, whose usage, if anything, decreased, and is accounted for, mostly, by increased use (or better reporting) by the University of California.

Thus our conclusion is, that, except for this one case, the trend for laboratory animal usage in the U.S. is essentially flat. From the available data, there is no justification for predicting either increases or decreases over the next several years. Were the resources available, it would be possible to examine the 1981 APHIS data sheets and determine whether on the basis of three years' data, a trend for the mandated species existed. The 1981 data sheets would not be useful for obtaining trends for rats and mice.

We do not believe that the other methods of estimating laboratory animal use are competitive with the use of the APHIS data, and thus do not lend much credence to the numbers reported in the past. It would be useful to be able to reconcile the results based on the APHIS data with the estimates based on animal breeder numbers. However, since information as to the distribution of costs per animal are proprietary, it is unlikely that this information can be obtained. It could conceivably be obtained by detailed surveys of users as to how many animals of what price range they each use. That information could be used to obtain a more reasonable estimate of the actual number of mice and rats bred and sold.

F. Recommendations for Future Study

The research and analysis for this report could have been substantially more thorough had more funding and time been available. But the major limitation was the need to depend on available data sources, with all their limitations as outlined in this report.

While the APHIS data sheets proved to be of considerable value, they still do not substitute for an appropriately designed stratified random sampling of all possible users. Only in that way does one really know whether all possible institutions have been included. The APHIS scheme depends on institutions to request certification. There could be institutions that are operating and are not reporting, because they are not included in the APHIS scheme. It would be possible, even, with some limitations, to do a post-hoc stratification based on the APHIS data, with considerable further effort.

Additional accuracy could be obtained for the rat and mouse estimates if resources were available to improve the regression equations used to estimate rat and mouse usage for those institutions that did not report the use of these species. Such things as careful examination of outliers, institution-specific equations, and a thorough diagnostic workup of the equations would undoubtedly produce still more reliable estimates.

Another area in which improvement could be made is in the assignment of institutions to the correct type. Limitations in resources necessitated a rather cursory assignment process, which could be much improved if additional effort could be spent on it.

In summary, then, laboratory animal use estimation could be improved further by the use of a stratified random sampling plan, improved institution type assignment, and the improvement of the regression equations for the estimation of rat and mouse use.

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Survey and Estimates of
Laboratory Animals Usage in the U.S.

-- Appendices --

Report Prepared for the
Office of Technology Assessment,
U.S. Congress

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27 July 1984

APPENDIX A

Estimate of an Average Cost for Purchase of Rats and Mice

For use in several extrapolation methods, an average cost for rats and mice was required. These figures were arrived at by speaking with several users and suppliers. This average was obviously intended to exclude exotic species and instead to represent a cross-section of the most common species of rats and mice used in laboratory experimentation in the U.S. If any doubt existed, the lowest possible (and seemingly appropriate) price was used. This permitted extrapolations based on price to represent an expected maximum.

Thus, the following were contracted to arrive at an average cost for rats:

- Charles River Breeding Laboratories (1983). Their cheapest weanlings costs $2.78, with prices ranging as high as $87.66. 22-30 day old rats range from $2.86-5.35; 31-35 days, $3.90-7.26, and so on, up to year-old rats at $9.57 to over $13.00. 21-day littermates range from $4.38-6.52 (for CD, Wistar, Long-Evans, Holtzman, CDF, Lewis or BN strains).

- Willett (1983), in his memo to the Director of NIH, Division of Research Resources, stated that in FY 1981, the average rat used "... at NIH (DRG) ..." cost $4.23 (ranging from $3.54 to $4.92).

- Norman Guilloud (1984), a researcher at Ortho Pharmaceutical Corporation, stated that the average cost of the rats they use is $5.25-5.50 and that that range is "... probably low ..." compared to others in the industry.

- Tony Miller (1984), a toxicologist at McNeil Laboratories, stated that their rats generally cost $5.00-9.00.

- Carol Kellogg (1984), a neuroscientist at the University of Rochester (NY) stated that her cost for male rats is typically $8.00-10.00.

Using these five sources, a cost of $4.00 per rat was estimated to represent a conservative (and if anything, probably low) price for a typical research rat.

Likewise, the following were consulted to obtain an average cost for mice:

- Charles River Breeding Laboratories (1983) sells the cheapest weanlings for $.92 and the most expensive C57BL/2 for $45.00. 22-29 day old mice sell for $.96-4.81, 29-35 day old mice for $1.03-5.08, and so on with 50 day olds costing from $1.11-5.61. 21 day old littermates range from $1.39-5.45.

- James Willett (1983) stated that, in FY 1981, the average mouse used "... at NIH (DRG) ..." cost $2.33 (ranging from $1.87-2.80).

- Norman Guilloud (1984), of Ortho Pharmaceutical, stated that the average cost of mice is $1.15-1.20, but that "... that range is probably low ..." compared to the rest of the industry.

- Tony Miller (1984), of McNeil Laboratories, said that mice generally cost $1.50.

- Eugene New (1984), Director of Laboratory Animal Science at the National Cancer Institute, stated that, of the more than 9 million mice issued in FY 81, 82 and 83, the average cost per animal was $3.95.

- Carroll Kellog (1984), of the University of Rochester stated that the average cost of a mouse used in teaching at the University is roughly $4.00.

With these six sources in mind, a cost of $2.00 per mouse was estimated to represent a conservative price for a typical mouse used in research in the U.S.

APPENDIX TABLE B-1

Regression Equation Coefficients and Statistics Based on Institutions Reporting Rat Usage

	Coefficients	
	1982	1983
Log # dogs	.159	.118
Log # guinea pigs	.192	.160
Log # hamsters	.231	.131
Log # rabbits	.286	.322
Log # primates	.287	.241
(Log cats - av log cats)^2	-.061	0
(Log hamsters - av log hamsters)^2	-.119	-.050
(Log rabbits - av log rabbits)^2	.055	.048
Universities	-.317	0
Medical schools	0	.160
Hospitals	-.348	-.277
For-profit biomedical research	-1.259	-.664
Toxicology testing laboratories	-.605	-.469
Chemical manufacturers	.374	.480
Pharmaceutical manufacturers	-.594	-.923
Devices and diagnostics manufacturers	-1.602	-1.570
Miscellaneous commercial	-1.249	-1.387
State and local governments	-1.080	-.492
Federal government	0	.169
Constant	1.600	1.460
N	1,014	1,108
R^2	.38	.35
Standard error of estimate	1.07	1.07

APPENDIX TABLE B-2

Regression Equation Coefficients and Statistics Based on Institutions Reporting Mouse Usage

	Coefficients	
	1982	1983
Log # guinea pigs	.360	.246
Log # hamsters	.203	.188
Log # rabbits	.446	.356
Log # primates	.206	0
$(\text{Log \# cats - av log \# cats})^2$	0	.055
$(\text{Log rabbits - av log rabbits})^2$	-.130	-.061
$(\text{Log primates - av log primates})^2$	0	.073
Universities	-.293	0
Medical schools	-.260	0
Hospitals	-.492	-.322
For-profit biomedical research	-1.060	0
Toxicity and testing labs	-.688	-.362
Chemical companies	0	.301
Devices and diagnostics manufacturers	-.429	-.679
Miscellaneous commercial	0	-1.004
State and local governments	0	.584
Constant	1.418	1.580
N	1,012	1,098
R^2	.43	.33
Standard error of estimate	1.21	1.20

Appendix C

Listing of the USDA/APHIS
Annual Reports of Research Facilities
by reporting institution*

- 1982 and 1983 -

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* Institution codes are listed at the end.

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
DC	1	1	-	-	-	39	92	-	-	-
DC	2	-	-	-	-	500	-	-	-	-
DC	3	215	564	21	-	19	49	16	-	-
DC	4	7,031	10,411	34	66	138	334	818	23	28
DC	5	8,977	9,206	180	343	370	243	83	3	-
DC	6	-	-	5	-	-	-	-	-	-
DC	7	-	-	-	-	-	-	-	-	-
DC	9	5,038	2,103	83	238	215	169	187	7	-
11	1	-	-	-	-	4,555	-	-	-	-
11	2	128	50	-	-	45	-	3	-	-
11	3	7	161	-	-	3	-	-		-
11	6	600	175	25	125	56	-	-	-	-
11	8	75	-	-	-	5	-	-	-	-
11	9	-	-	-	173	-	-	-	-	-
11	10	-	-	-	-	20	-	-	-	-
11	11	-	-	13	-	50	-	-	-	-
11	12	-	-	-	-	28	-	-	-	-
11	13	-	-	5	-	181	-	-	-	-
11	14	-	158	-	-	-	-	-	-	-
12	1	-	-	15	806	439	77	540	-	-
12	2	85	-	-	-	-	-	-	-	-
12	3	123	410	-	3	38	-	-	-	65
13	1	8,717	10,590	591	262	1,218	59	26	12	-
13	3	-	-	-	-	12	-	-	-	-
13	5	3	2	8	15	-	-	-	-	-
13	9	-	-	-	-	-	2	5	-	-
14	1	-	-	-	-	52	-	-	-	-
14	3	804	32,246	-	-	258	-	-	-	-
14	4	197	-	-	-	-	-	-	-	-
14	5	-	-	-	7	69	28	-	3	-
14	6	-	-	120	1,905	1,120	18	-	163	-
14	8	5,585	1,022	153	-	494	32	-	-	-
14	10	594	1,236	154	-	1,785	-	17	136	-
14	11	10	-	120	-	-	-	-	-	-
14	12	7,307	1,226	-	1,655	36	-	-	35	-
14	13	800	4,000	30	-	96	70	-	-	-
14	14	19,529	21,337	707	408	2,673	2,186	569	53	-
14	15	-	-	1,364	110	912	363	5	8	-
14	17	-	-	260	102	2,905	207	4	151	-
14	19	15,834	104,581	2,305	1,984	1,561	2,310	573	498	-
14	20	-	-	222	48	865	67	19	34	-
14	21	3,232	5,343	63	-	156	-	-	32	40
14	22	-	-	122	130	289	4	374	15	-
14	23	1,560	1,820	205	-	430	27	-	-	-
14	24	-	-	-	11	-	-	-	-	-
14	25	3	4	2	2	1	4	5	-	-
14	30	425	12,095	-	30	141	-	-	-	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
14	32	2,214	858	225	-	256	-	-	-	-
14	33	2,970	7,001	108	52	192	-	-	-	-
14	35	89	6,466	329	521	1,461	347	28	28	-
14	37	2,182	1,073	-	153	8	-	8	-	-
14	38	208	2,175	-	-	75	-	-	-	-
14	44	7,647	16,885	520	504	300	58	-	-	-
14	45	-	-	-	-	-	20	-	-	-
14	50	-	-	-	1,623	5	-	-	-	-
14	54	-	-	-	3,540	-	-	-	-	-
14	61	-	75	-	-	6	-	-	-	-
14	64	-	-	-	-	119	-	-	-	-
14	68	-	250	-	-	-	-	-	-	-
14	69	-	-	6	-	47	-	-	-	-
14	73	-	-	4	-	3	280	204	-	-
14	75	-	-	-	-	54	-	-	-	-
14	77	-	-	-	-	17	-	-	-	-
14	80	-	-	140	20	1,371	6	-	-	-
14	81	-	1,600	-	800	-	-	-	70	-
14	82	8,968	36,160	272	209	1,285	180	27	49	23
14	83	-	-	368	121	2,859	-	-	-	-
14	84	1,610	2,018	-	304	189	-	-	36	-
14	86	1,000	300	20	-	170	-	-	-	-
14	87	-	-	-	-	6	-	-	2	-
14	88	120	20	-	40	-	-	-	-	-
14	89	511	390	-	-	153	-	-	-	-
14	92	5,426	270	405	-	215	13	-	-	-
14	93	-	-	6	762	575	-	2	-	-
14	94	-	-	-	-	3	-	-	-	2
14	95	-	-	140	-	-	-	-	-	-
14	96	-	-	-	-	39	-	-	26	-
14	97	-	-	-	-	-	21	-	-	-
14	98	-	-	-	-	-	20	-	-	-
14	99	46	-	-	-	7	-	-	-	-
15	2	-	-	299	-	87	189	514	-	-
15	3	-	-	-	42	78	47	65	152	-
15	4	504	330	124	-	24	-	-	9	-
15	5	-	-	-	-	-	125	-	-	-
15	6	-	-	-	30	9	23	-	-	-
15	10	-	-	-	-	6	-	-	-	-
15	14	-	-	-	-	18	-	-	-	-
16	1	-	-	2,929	1,046	3,379	732	465	34	-
16	2	551	-	-	-	-	-	-	-	-
16	4	440	7	260	-	47	-	-	28	-
16	7	-	-	-	-	10	69	-	7	-
16	8	-	-	13	-	398	207	48	8	-
16	11	307	-	-	20	4	-	-	-	-
16	16	-	-	-	-	3	-	-	-	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
16	23	79	-	-	-	40	-	-	-	-
16	26	-	-	4	4	10	8	7	-	-
16	28	-	-	-	-	-	644	-	-	-
16	29	-	-	2,826	160	49	49	8	17	-
16	32	12,373	4,034	-	-	205	92	-	32	-
21	1	32	40	-	-	28	70	-	-	-
21	2	132	-	-	-	-	-	-	-	-
21	3	-	-	2,126	-	265	727	664	-	-
21	7	334	302	101	76	2	-	-	-	-
21	8	160	1,980	346	-	730	-	-	-	-
21	11	-	-	17,066	807	995	1,799	316	432	-
21	12	-	-	149	-	31	-	-	-	-
21	15	3,608	-	10	120	85	9	-	7	-
21	17	-	227	20	-	44	59	67	-	-
21	22	-	-	150	-	609	425	-	-	-
21	24	-	-	22,581	-	3,971	653	26	1,188	-
21	28	870	-	5	-	-	43	239	-	-
21	33	199	284	-	259	108	109	53	-	-
21	34	-	-	777	311	475	-	-	-	-
21	36	-	-	84	944	1,234	118	364	123	107
21	37	-	-	-	-	-	124	80	-	-
21	38	-	-	-	-	248	8	-	-	-
21	40	11,801	13,746	307	1,691	1,537	343	18	43	-
21	42	-	-	40	-	-	16	-	-	-
21	43	-	-	1,319	-	1,180	-	-	-	-
21	45	200	320	110	-	90	-	-	-	-
21	46	120	-	-	-	2	-	-	-	-
21	48	-	-	892	402	662	235	28	27	-
21	50	-	-	2,586	-	2,880	-	-	-	-
21	54	200	85	-	-	-	-	-	-	348
21	58	-	-	-	-	20	-	-	-	-
21	59	70	200	-	-	96	90	-	-	-
21	60	-	-	98	115	584	266	161	-	-
21	61	-	-	-	-	7	-	-	-	-
21	62	544	-	-	-	20	-	-	31	-
21	63	100	90	-	-	-	-	-	-	-
21	64	20	500	-	-	14	-	-	-	-
21	66	-	-	-	-	-	12	-	-	-
21	67	748	-	-	-	-	-	-	-	-
21	69	1,462	848	1	25	257	307	-	-	-
21	70	-	-	48	-	12	-	-	-	-
21	71	3,459	24	415	42	185	78	-	5	-
21	72	-	-	152	299	204	48	120	7	-
21	73	948	12	28	-	22	111	8	-	-
21	74	2,253	4,318	-	-	24	254	20	6	-
21	76	210	25	92	-	14	24	-	-	-
21	79	480	-	-	-	-	-	-	27	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
21	81	-	-	64	44	264	334	12	-	-
21	86	90	300	-	12	-	-	-	-	-
21	87	12,834	571	3,699	-	476	11	-	-	-
21	88	-	-	9,491	843	1,225	1,883	55	6	-
21	89	-	-	32	-	-	-	72	-	-
21	91	50	-	-	-	1	-	-	-	-
21	96	-	-	-	35	2	-	-	-	-
21	98	-	-	-	-	12	-	-	-	-
21	100	-	-	-	6	-	-	-	-	-
21	103	-	-	62	72	401	331	805	19	-
21	104	9,570	9,044	224	438	1,442	1,041	123	121	-
21	106	-	-	-	-	6	-	-	-	-
21	107	2,294	117	6	182	15	103	-	-	-
21	109	22,094	20,204	124	134	505	743	145	130	-
21	110	-	-	15	251	67	-	-	-	-
21	112	600	30	-	-	5	41	-	-	-
21	114	29	-	729	821	1,441	333	286	7	-
21	115	-	-	-	-	53	218	-	-	-
21	116	695	1,647	20	-	102	69	-	-	-
21	117	14	-	-	-	-	95	16	-	-
21	118	18,710	40,817	967	1,580	1,010	262	473	457	3,588
21	119	58	19	272	251	559	1,624	554	11	-
21	122	-	-	2	-	30	-	-	-	-
21	123	269	-	-	-	102	84	-	-	-
21	124	2,652	45,920	1,943	860	620	187	13	63	-
21	125	-	-	-	-	16	135	-	-	-
21	126	688	260	12	5	22	-	-	-	-
21	127	-	-	1,104	54	1,774	79	77	-	-
21	128	574	2,750	10	-	87	-	-	-	32
21	129	10	-	-	-	15	70	6	15	-
21	130	122	2,700	22	-	48	101	-	1	6
21	131	2,000	5,000	-	-	920	275	50	-	-
21	132	-	-	1,051	426	503	620	572	5	-
21	133	7,670	22,144	924	378	368	-	74	92	64
21	134	-	-	140	-	321	-	-	28	50
21	135	-	-	150	50	7	-	-	-	-
21	136	23,870	33,881	1,851	1,343	3,156	1,263	1,142	453	68
21	137	2,137	61	-	-	112	71	-	-	45
21	138	400	1,900	-	-	15	22	33	-	-
21	139	-	-	-	-	273	170	-	20	-
21	140	-	-	26	-	145	58	2	-	-
21	141	98	-	-	-	49	-	-	-	-
21	142	2,123	2,685	2	231	50	3	3	21	-
21	146	-	123	14	48	7	-	-	-	-
21	148	241	400	-	-	30	-	-	-	-
21	151	27	99	12	-	17	-	-	-	-
21	152	325	-	-	-	-	-	-	-	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
21	153	2,734	490	326	-	442	-	-	-	-
21	154	-	-	1,592	-	-	-	-	-	-
21	159	-	-	-	-	50	-	-	-	-
21	162	-	-	-	-	-	-	-	66	-
21	164	3,305	302	5,810	610	1,510	67	-	-	-
21	165	-	-	32	-	124	-	-	-	-
21	166	110	423	-	-	25	-	-	-	-
21	167	84	1,239	-	-	53	-	-	-	-
21	168	-	2,601	-	-	104	-	-	-	-
21	169	-	-	-	-	-	6	-	-	-
21	170	-	-	-	-	8	-	-	-	-
22	5	-	6,605	-	-	-	-	-	52	18
22	9	-	-	404	20	1,294	558	2	526	-
22	10	3,930	10,509	-	-	2,742	159	-	-	-
22	11	1,165	580	278	-	2,460	-	-	-	-
22	12	15,321	120	-	-	317	232	-	-	-
22	14	-	-	2,301	-	1,110	925	-	8	-
22	16	13,245	11,280	7,250	-	1,788	205	-	-	-
22	20	-	-	455	241	226	483	249	54	-
22	21	-	-	265	-	906	55	-	-	-
22	22	1,780	1,500	-	1,100	43	-	60	6	-
22	25	11,440	12,565	1,881	1,407	1,332	26	26	29	1,418
22	26	-	-	48	-	842	-	14	-	-
22	28	28,212	163,072	1,506	154	1,813	588	120	26	-
22	29	3,400	200	-	47	58	57	-	-	-
22	30	-	-	8,820	3,355	9,276	1,580	236	766	-
22	31	-	-	-	-	-	70	1	-	-
22	32	-	-	11,436	1,725	2,101	1,499	136	372	-
22	33	-	-	2,392	124	4,464	705	-	32	-
22	36	-	-	12,100	1,250	1,361	1,351	104	261	-
22	37	37	2,580	-	-	8	-	-	-	-
22	39	-	-	-	-	44	-	-	-	-
22	41	31	7,000	-	-	2,262	17	-	-	-
22	42	-	-	119	-	178	466	-	4	-
22	44	57	29,997	190	6,040	77	336	157	-	-
22	45	-	-	-	-	17	-	-	-	504
22	49	40	-	64	-	28	-	-	-	-
22	50	-	300	-	-	66	-	-	-	-
22	55	-	71	-	-	61	-	-	-	-
22	58	-	-	755	63	3,357	87	-	-	-
22	60	-	-	-	-	108	-	-	-	-
22	63	2,051	509	24	-	1,431	-	-	-	-
22	64	7	1,064	152	-	2,037	-	-	-	-
22	65	-	-	-	-	-	-	-	-	-
22	66	3,550	3,196	44	1,470	981	290	119	-	-
22	67	-	490	4	-	16	-	-	-	-
22	69	6,683	144	1,694	-	3,185	-	-	-	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
22	71	-	-	2,704	-	781	-	-	-	-
22	72	142	580	-	-	16	-	-	-	-
22	73	126	-	-	-	-	-	-	-	-
22	74	3,476	1,231	850	100	793	-	-	-	-
22	75	494	-	133	-	165	-	-	-	-
22	76	80	10	4	-	4	3	2	-	-
22	77	-	-	20	-	413	-	-	-	-
22	78	50	50	50	-	50	-	-	-	-
22	80	95	-	90	-	-	612	39	-	-
22	82	-	-	200	-	1,200	-	-	-	-
22	85	-	-	-	-	-	-	2	-	-
22	86	-	-	-	-	-	109	-	-	-
22	87	2,945	17,315	553	-	4,572	13	-	-	-
22	88	-	-	170	-	594	10	-	-	-
22	89	8,896	1,756	-	-	-	1	72	-	-
23	1	1,618	8,188	310	100	102	8	9	-	-
23	3	367	72	-	-	295	230	-	-	-
23	5	5,203	4,114	1	-	516	108	209	-	-
23	6	-	-	-	-	-	104	-	-	-
23	8	-	-	50	-	40	-	-	-	-
23	9	25,443	12,855	1,030	15	44	287	-	4	-
23	10	100	-	-	-	-	-	-	-	-
23	11	5,298	21,384	634	364	1,623	206	663	33	-
23	12	-	-	7,619	19,322	18,842	1,575	897	705	-
23	13	288	-	4	-	16	-	-	-	-
23	15	80,286	134,476	7,236	320	216	975	-	112	-
23	16	20,803	25,346	590	12	1,428	770	257	360	-
23	18	41,164	139,253	8,586	433	2,473	800	314	28	-
23	19	233	5	108	-	28	296	13	-	-
23	21	35,645	20,955	689	1,183	2,197	231	54	186	80
23	22	754	29,511	-	-	64	49	-	-	-
23	23	27,350	30,941	1,056	1,626	2,918	1,039	836	55	5
23	24	3,550	3,644	288	-	7,084	-	-	-	-
23	25	150	150	5	-	6	145	24	-	-
23	26	40	-	11	-	-	17	6	-	-
23	27	20,840	20,071	608	599	537	472	592	-	-
23	29	1,107	1,066	6	-	192	-	53	-	-
23	31	8,177	21,154	329	671	167	445	293	330	-
23	32	60	495	-	14	21	60	6	-	-
23	33	6,279	13,742	178	-	197	44	100	-	242
23	35	19,520	33,900	335	-	88	157	-	18	-
23	37	10,630	8,831	471	-	1,650	136	-	-	-
23	42	650	254	22	-	186	-	-	-	-
23	45	3,622	3,035	70	-	-	72	-	-	-
23	46	330	190	-	731	18	-	-	8	-
23	47	-	-	-	-	-	6	-	-	-
23	48	1,500	-	-	-	-	-	11	-	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
23	49	20	550	-	-	527	-	-	-	-
23	50	251	90	-	-	-	-	-	-	-
23	52	-	30	-	-	-	-	-	-	-
23	54	-	2,145	140	50	-	-	-	-	-
23	55	3,394	52,202	48	651	346	-	-	-	-
23	56	-	-	-	-	-	-	-	13	-
23	57	6	200	-	-	-	-	-	-	-
23	58	384	904	20	-	31	-	-	-	-
23	60	-	5	-	-	26	-	-	-	-
23	64	32	500	-	-	37	-	-	-	-
23	65	-	-	-	-	-	269	40	-	-
23	67	1,312	42,654	-	-	41	1,116	6	467	-
23	69	810	1,026	24	-	48	28	-	-	-
23	75	6	2	4	-	-	20	16	-	-
23	76	173	246	4	45	1	-	-	-	-
23	78	-	-	70	125	17	15	16	-	-
23	82	10,600	550	2,803	-	5,117	-	12	-	-
23	84	40	41,305	2,127	-	504	-	-	-	-
23	85	144	4,065	-	-	11	23	-	-	-
23	87	-	270	-	-	188	-	-	-	-
23	88	876	1,680	230	13	-	-	-	200	80
23	90	500	-	-	-	-	-	2	37	-
23	91	411	464	-	-	36	-	-	60	-
23	92	2,237	4,571	50	500	258	143	-	50	1
23	94	600	50	-	-	58	1,706	96	-	-
23	96	18	462	25	-	180	-	-	-	-
23	97	602	-	-	-	394	-	-	-	-
23	99	5,360	-	-	-	1,085	-	-	-	-
23	101	-	-	-	-	-	216	-	-	-
23	102	129	6	-	-	16	-	-	-	-
23	103	551	160	-	-	14	-	-	-	-
23	106	-	-	-	-	-	-	-	-	-
23	107	7,408	6,045	912	12	1,992	-	-	-	-
23	108	-	-	34	40	124	1	-	-	-
23	109	2,243	4,196	41	-	665	-	-	-	-
23	110	-	-	-	-	22	-	-	-	-
31	6	220	290	-	30	24	-	-	-	56
31	9	-	-	3,494	-	1,170	-	-	-	-
31	10	-	-	4,122	848	1,732	171	-	-	-
31	11	1,255	2,936	29	-	57	250	22	40	44
31	13	-	-	-	-	415	49	12	-	-
31	14	-	-	813	861	1,557	1,926	558	76	-
31	16	3,661	550	1,192	-	1,087	25	-	-	-
31	17	-	-	140	-	545	690	166	-	-
31	19	301	320	9	-	-	6	-	-	-
31	21	6,953	359,293	896	-	101	83	-	-	-
31	25	435	6	10	7	54	432	2	-	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
31	26	1,162	1,157	110	-	6	11	24	-	-
31	27	-	-	1,174	698	1,003	1,482	430	52	-
31	28	9,258	18,087	728	879	1,255	734	434	46	-
31	29	-	1,320	34	-	5	-	-	-	-
31	30	2,998	1,365	90	-	76	-	-	9	-
31	31	17	30	-	-	6	118	-	-	-
31	33	97	-	-	-	42	200	-	-	-
31	35	100	955	-	-	7	-	-	-	-
31	40	852	1,348	30	-	50	10	-	-	-
31	43	1,830	8,298	160	12	239	575	133	5	-
31	47	-	-	-	-	49	-	-	-	-
31	49	355	138	-	8	2	-	-	5	-
31	50	212	40	-	-	-	-	-	-	-
31	53	-	-	4	-	12	-	4	5	-
31	54	341	12	-	-	21	-	-	-	-
31	55	3,050	5,304	65	269	98	259	77	-	-
31	57	-	-	-	-	-	324	-	-	-
31	58	75	2,000	26	4	18	-	-	-	-
31	59	-	-	909	-	-	-	-	-	-
31	61	104	231	-	-	3	33	-	-	-
31	63	-	-	-	-	4	9	-	-	-
31	65	500	140	-	-	-	27	-	-	-
31	68	877	26,016	381	-	5,068	25	-	-	-
31	70	-	475	-	-	-	-	-	-	-
31	78	140	-	-	-	-	-	-	-	-
31	79	953	-	24	55	66	-	-	-	75
31	80	-	20	-	-	-	18	-	-	-
31	82	-	9	13	2	165	15	110	-	-
31	84	266	14	485	257	378	870	113	77	-
31	86	-	1,160	4	-	-	-	-	-	-
31	87	110	176	25	-	46	5	83	-	-
31	88	4	4	2	-	2	12	7	-	-
31	89	-	-	967	-	2,634	215	-	-	-
31	90	4,792	20,275	486	-	92	62	22	-	-
31	91	5,295	16,360	42	-	-	-	-	-	-
31	92	3,139	5,868	3	1,105	126	434	279	15	-
31	93	-	60	10	-	54	-	-	-	-
31	95	110	160	-	-	-	-	-	-	-
31	96	-	-	-	-	41	-	-	-	-
32	1	3,735	635	2,368	-	1,845	352	13	38	-
32	2	-	-	-	-	-	15	-	-	-
32	3	-	-	11,120	639	3,904	2,083	1,876	77	-
32	4	56	7	-	-	-	-	-	-	-
32	5	-	-	2,573	-	560	1,398	64	32	-
32	6	38,463	23,321	1,625	485	1,074	2,402	424	96	29
32	7	-	-	291	300	779	786	8	21	-
32	9	600	-	-	-	82	260	20	-	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
32	10	300	1,000	-	-	4	4	-	-	-
32	11	-	-	5	176	3	-	-	-	-
32	14	443	21	2	-	4	-	-	-	-
32	16	50	200	8	50	11	3	27	-	-
32	19	-	-	6	-	60	-	-	-	-
32	20	3,651	108	80	-	783	26	-	-	-
32	23	-	-	116	-	409	-	-	-	-
33	3	265	1,300	-	36	87	-	-	-	-
33	6	607	141	-	-	755	30	11	-	-
33	8	7,743	337,187	520	6,865	1,122	-	-	-	1,000
33	9	3,441	7,942	94	524	179	297	-	44	322
33	10	1,200	5,000	-	-	42	59	19	-	-
33	13	575	992	-	50	35	112	-	1	-
33	14	9,992	9,816	316	74	629	270	167	10	-
33	18	1,638	156	980	2,048	2,580	870	325	644	-
33	20	1,395	9,528	22	19	173	614	-	-	-
33	22	-	-	657	95	911	293	23	65	-
33	23	5,160	13,857	10,973	-	7,959	207	5	-	-
33	24	12,863	8,629	784	553	936	1,204	226	22	-
33	25	-	-	1,192	420	7,619	552	64	764	-
33	26	808	28	4	27	114	7	-	-	-
33	27	-	-	192	-	120	202	-	-	-
33	28	-	-	5,740	1,660	928	1,256	83	247	-
33	29	-	-	225	2,164	680	810	315	27	-
33	30	45,711	26,810	505	137	1,438	1,216	25	261	152
33	31	-	-	-	-	-	486	-	-	-
33	33	56,801	3,479	3,501	-	8,811	9	-	-	-
33	34	1,349	1,295	1,559	-	710	1,240	101	-	-
33	35	300	2,000	30	-	150	200	22	30	-
33	36	-	-	-	-	40	-	-	39	-
33	38	-	-	-	-	62	50	-	-	-
33	40	23,328	32,025	465	2,888	1,312	780	334	91	-
33	41	-	-	-	-	-	59	-	-	-
33	43	30	220	-	-	887	-	-	-	-
33	44	367	293	-	-	18	-	-	-	-
33	45	131	-	-	200	31	-	-	-	-
33	47	2,500	-	-	-	-	12	-	-	-
33	48	-	-	-	-	-	5	-	-	-
33	49	30	-	-	-	-	-	-	-	-
33	51	10	-	8	8	2	-	-	-	-
33	52	48	-	-	-	-	-	23	-	-
33	54	8	-	-	-	-	-	-	-	-
33	56	480	-	83	-	-	-	-	-	-
33	58	-	-	-	-	36	-	-	-	-
33	59	120	100	-	-	4	-	-	-	-
33	62	750	200	-	-	10	-	-	-	-
33	67	2,128	736	-	73	8	-	-	-	250

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
33	68	730	141	-	-	84	22	-	-	-
33	69	1,138	1,140	-	-	-	-	-	9	-
33	75	-	-	-	-	78	232	65	-	-
33	77	-	-	-	-	-	4	-	-	-
33	79	780	300	-	-	6	-	-	-	-
33	81	-	-	-	-	-	223	274	-	-
33	83	50	100	-	-	9	-	-	-	-
33	84	-	7,826	-	-	-	-	-	-	-
33	87	-	-	-	-	66	-	-	-	-
33	88	-	-	-	-	-	-	-	-	-
33	89	-	-	-	-	-	-	-	-	-
33	90	-	-	150	-	1,525	-	-	-	-
33	91	12,099	7,910	452	57	1,198	88	-	-	-
33	93	-	-	-	-	55	-	-	-	-
33	94	176	183	31	-	740	-	-	-	-
34	1	46,019	52,994	1,689	1,989	2,767	2,190	440	373	-
34	3	19,901	4,055	166	-	3,946	810	-	300	-
34	5	34,813	114,346	6,421	64	519	1,423	9	94	-
34	6	-	-	-	-	19	100	-	-	-
34	7	38	-	-	-	-	46	4	-	-
34	8	43,587	112,649	11,029	54	2,680	623	11	321	-
34	9	605	320	-	47	74	6	6	-	165
34	10	6,195	3,909	24	-	200	397	41	21	100
34	11	844	3,645	-	-	-	-	-	13	-
34	12	300	200	-	-	4	304	12	-	-
34	14	-	-	109	634	794	604	38	41	-
34	15	101,395	274,046	5,529	3,892	3,320	821	322	115	-
34	16	-	-	-	-	-	38	-	-	-
34	17	-	506	3,419	1,026	339	1,500	567	152	84
34	18	34	242	3	-	24	59	-	-	-
34	19	1,230	200	-	-	60	3	2	-	-
34	20	10	-	-	20	-	-	-	-	-
34	21	290	1,400	20	-	-	-	-	-	-
34	24	75	100	32	80	6	-	-	-	-
34	27	-	56,673	5,665	6,316	624	-	-	49	-
34	28	-	-	-	-	-	30	-	-	-
34	32	1,375	2,370	705	-	836	8	-	-	-
34	34	-	-	-	-	-	395	72	-	-
34	35	250	60	12	96	-	-	-	-	-
34	36	83	454	-	-	23	31	5	-	-
34	37	65	715	10	263	54	48	4	-	4
34	38	-	-	23	-	1,293	97	8	-	-
34	39	500	3,750	6	-	12	-	-	-	-
34	40	50	-	-	-	-	-	-	-	-
34	42	10	1,259	-	-	-	-	-	-	-
34	43	-	-	-	-	39	-	-	-	-
34	44	342	230	-	-	7	-	-	-	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
34	45	17	-	-	-	-	-	-	-	-
34	47	900	700	-	-	6	1	-	-	-
34	50	-	-	-	-	-	-	-	6	-
34	51	8	-	-	-	-	-	-	-	-
34	52	2,184	1,223	262	23	-	53	-	-	-
34	54	3,394	2,412	2,205	-	1,106	85	-	-	-
34	55	-	-	-	4	-	-	35	-	-
34	57	-	-	-	-	-	4	-	-	-
35	1	-	1,000	2,371	6,008	1,865	1,251	801	1,606	1,794
35	8	-	-	-	-	90	-	-	6	-
35	9	1,858	33,224	-	112	594	162	-	-	-
35	11	1,784	4,018	72	200	168	-	-	-	-
35	12	-	38,330	200	7,750	576	574	548	-	-
35	15	2,500	250	-	-	-	-	-	-	-
35	16	-	-	-	-	-	70	52	-	-
35	20	-	187	43	-	-	-	-	-	-
35	21	50	30	-	30	17	-	-	-	-
35	22	-	-	-	-	-	8	-	-	-
35	23	-	-	-	-	-	8	8	-	-
35	24	32	100	-	-	51	-	-	-	-
35	27	-	2	7	-	-	-	-	-	-
35	29	6,687	10,325	351	158	1,382	1,176	251	67	-
35	30	-	-	1,405	-	4,232	50	24	39	-
41	3	415	550	24	-	8	228	36	-	-
41	5	17,697	35,081	1,923	866	4,088	3,251	1,026	34	390
41	6	7,129	96,859	1,611	24	1,338	2,473	538	33	-
41	7	1,436	-	12	-	20	181	2	-	-
41	8	4,190	18,580	5,106	45	2,890	107	114	3	-
41	10	14	9	-	-	-	-	-	-	-
41	11	172	6	-	-	18	-	-	-	-
41	12	10	-	-	-	-	-	-	-	-
41	14	358	149	2	-	6	-	-	-	-
41	15	2,713	2,896	-	-	162	250	-	-	-
41	18	2	400	40	-	-	-	-	-	-
41	19	-	-	-	-	459	-	-	-	-
41	21	-	-	-	-	-	33	-	-	-
41	22	21	-	-	-	-	196	-	-	-
41	24	-	-	-	-	99	-	-	-	-
42	1	-	-	1,974	11,591	493	-	-	-	-
42	2	36	20	-	-	5	317	25	-	-
42	3	2,945	15,000	552	2	512	616	234	60	-
42	4	-	-	1,172	767	4,296	2,454	847	128	-
42	5	145	-	-	-	12	25	-	-	25
42	7	320	5,225	516	100	638	-	4	-	-
42	9	-	6,335	128	1,005	165	95	56	-	-
42	11	200	50	-	-	30	-	-	-	-
42	12	-	-	711	225	284	-	-	-	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
42	13	-	-	-	-	-	1	-	-	-
42	15	-	2,975	12	-	4	-	-	-	-
42	16	-	-	-	-	-	8	-	-	-
43	1	128	20	-	-	258	29	-	-	-
43	2	-	-	64	-	924	318	21	8	-
43	3	130	100	-	-	6	-	-	-	-
43	5	-	-	282	-	776	123	-	-	-
43	6	1,253	-	40	-	196	1,938	1,194	-	-
43	7	1,972	19,220	-	234	171	154	-	-	-
43	8	-	-	3,904	2,910	8,382	1,960	794	222	-
43	9	-	-	23	-	166	60	-	-	-
43	10	-	132	8,938	2,900	2,969	3,830	1,084	684	-
43	11	10,554	10,680	47	253	447	459	178	2	-
43	14	-	110,950	4,647	18,432	2,412	1,074	538	-	-
43	15	-	-	-	-	301	-	-	-	-
43	21	53	-	-	-	103	-	-	-	-
43	23	-	-	-	-	91	-	-	-	-
43	24	3,318	683	159	-	211	44	-	-	-
43	25	-	-	-	-	135	-	-	-	-
43	26	-	-	-	-	1,100	-	-	-	-
43	29	-	-	5	3	1	28	18	-	-
43	30	-	4	8	4	2	118	22	-	-
43	31	84	50	28	48	42	84	-	-	-
43	33	19,586	1,912	62	-	1,888	369	-	-	-
43	35	-	-	-	-	-	52	-	-	-
43	36	-	-	-	-	128	-	-	-	-
45	1	458	1,320	22	-	318	200	36	-	37
45	2	3,810	6,940	57	39	47	65	18	-	19
45	3	-	-	-	-	-	-	-	-	-
46	1	1,600	2,400	154	764	132	90	20	-	-
46	2	24	850	20	-	42	11	-	-	-
47	2	639	5,282	168	72	195	431	42	-	-
47	4	16,502	14,733	106	4,207	1,506	323	51	45	407
47	5	-	31,297	1,633	10,675	939	516	177	-	-
47	7	20	120	-	-	7	-	-	-	-
47	8	6	26	-	-	-	-	-	-	-
47	9	-	-	-	-	-	-	30	-	-
47	10	-	37,500	273	1,022	352	41	101	-	-
47	11	-	24	-	-	-	-	-	-	-
47	13	-	-	-	-	3	9	3	-	-
46	1	6	30	133	427	218	570	31	6	-
48	2	8,123	9,381	75	64	729	9	24	-	-
48	3	-	35	77	4,063	1,417	321	242	11	-
48	4	-	-	220	-	422	60	-	-	-
48	5	-	-	4,651	5,050	1,574	125	89	-	-
48	9	-	-	-	-	-	5	-	6	-
48	11	-	13,800	138	-	138	270	170	-	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
48	14	-	-	-	45	4	-	-	-	-
48	15	4	95	5	-	50	-	-	-	-
48	17	66	200	-	-	11	-	-	-	-
48	18	-	-	-	-	12	-	-	-	-
48	19	-	12,150	200	4,885	121	90	90	-	-
48	20	36	-	24	-	-	-	-	-	-
48	22	-	2,200	20	-	20	30	-	-	-
50	1	21,210	6,997	1,736	112	1,144	475	-	3	-
50	3	64,185	97,062	3,752	2,060	818	304	15	109	-
50	4	1,800	1,500	43	15,000	97	26	28	16	-
50	7	-	782	-	-	38	-	-	-	-
50	8	555	1,543	6	-	-	-	10	-	-
50	9	6,052	46	-	-	198	-	-	-	-
51	3	3,731	31,847	4	1,024	36	-	-	-	-
51	6	55,535	46,315	876	1,811	4,620	3,479	160	119	-
51	11	-	-	9	12	24	55	16	-	-
51	12	50	-	-	-	-	4	-	-	-
51	14	3,528	13,551	1,005	371	294	84	13	68	17,915
51	15	580	580	-	-	20	192	-	-	-
51	16	3,000	-	-	-	-	-	-	-	-
51	20	117	12	1	-	2	7	5	-	-
51	21	2	24	-	-	257	-	-	-	10
51	24	-	4,000	-	-	9	-	-	-	-
51	25	610	3,088	58	2,018	1,088	-	-	-	-
51	26	2,465	559	10	-	12	-	-	-	-
51	27	4,015	3,073	-	973	262	380	-	-	-
51	28	-	7,800	-	-	108	-	-	-	-
51	32	1,000	31,586	10,200	-	-	-	-	-	-
51	33	-	-	-	-	16	-	-	-	-
51	34	34	143	-	-	41	-	-	-	-
51	36	-	-	-	-	54	-	-	-	-
51	37	-	-	-	-	-	-	-	16	-
52	5	-	843	50	260	145	184	24	-	-
52	6	-	-	486	50	2,379	761	-	1,102	-
52	7	-	-	6,050	330	1,219	1,435	1,612	225	-
52	9	437	1,402	39	-	97	216	3	14	149
52	10	13,203	-	51	543	800	3	62	410	-
52	11	20,876	23,161	1,249	750	2,347	568	273	69	-
52	13	450	500	-	-	90	-	4	-	-
52	14	-	-	-	-	3	-	-	-	-
52	17	-	-	-	-	-	8	5	-	-
52	19	835	100	-	10	6	-	-	-	-
52	20	30	-	-	-	3	159	-	-	-
52	21	50	200	2,155	-	310	-	-	425	-
52	23	404	50	-	-	3	-	-	-	-
54	1	13,717	3,679	1,269	329	663	385	91	15	-
54	2	1,000	500	-	-	4	50	10	6	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
54	3	6	-	2	-	-	48	24	-	-
54	4	2,722	638	-	-	15	17	12	-	-
54	5	-	-	-	-	-	6	-	-	-
55	1	-	-	848	-	1,396	588	86	1,555	-
55	2	-	-	26	26	627	3	-	6	-
55	3	-	-	1,243	207	2,351	1,784	540	23	-
55	4	225	-	787	917	1,914	1,306	594	39	-
55	5	-	3,280	54	347	175	38	18	-	-
55	7	-	-	-	-	24	-	-	-	145
55	9	-	-	10,987	8,571	2,023	1,267	583	-	-
55	10	-	-	581	546	322	223	72	41	-
55	12	-	-	-	-	-	-	-	20	-
55	13	-	-	2,002	-	47	-	-	-	-
55	16	43	9	-	-	3	-	-	-	-
55	18	-	-	-	-	-	161	-	-	-
55	19	-	-	15	1	2	40	30	-	-
55	20	-	-	-	-	64	-	19	-	-
56	1	-	-	12	1,613	1,006	1,076	134	36	-
56	2	-	-	53	5	108	89	2	-	25
56	3	4,156	3,427	33	24	227	174	79	-	-
56	4	-	-	3	-	6	60	30	-	-
56	5	54	20	-	-	-	-	-	-	-
56	6	-	-	255	-	-	-	-	-	-
57	2	15,447	7,641	340	1,784	4,856	327	46	58	-
57	3	9,205	11,221	277	125	2,602	759	218	2,235	-
57	4	18	-	-	-	-	-	-	-	-
57	5	7,795	28,277	158	42,292	590	1,656	368	246	40
57	10	345	428	-	-	22	19	-	-	-
57	12	211	-	-	-	24	-	-	-	40
57	13	4,891	1,078	16	278	644	18	1	-	400
57	14	-	-	-	-	-	-	210	-	-
58	1	-	-	4	81	84	57	142	12	-
58	2	-	-	88	-	786	50	-	6	-
58	3	-	840	505	1,973	1,895	1,840	639	101	-
58	5	-	-	-	35	38	25	-	-	-
58	7	-	-	299	162	1,837	232	256	412	-
58	9	-	-	-	40	30	81	-	-	-
58	12	-	-	-	-	12	25	-	-	-
58	14	-	-	-	-	2,209	-	-	-	-
58	15	-	-	108	876	91	945	237	12	-
58	16	-	-	-	-	25	-	-	-	-
58	17	-	-	-	20	20	1	-	-	-
58	19	-	6,500	1,300	-	690	-	-	-	-
58	20	600	1,175	-	21	55	-	-	-	10
58	21	-	-	-	-	-	-	-	632	-
58	22	31	24	-	2	-	3	-	-	-
58	23	-	-	-	-	-	7	15	-	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
58	24	-	-	-	-	48	-	50	-	-
58	25	-	-	256	-	850	-	-	-	-
58	28	-	400	-	-	2,050	-	-	-	-
58	29	-	-	-	-	-	-	-	-	3
58	30	-	-	2	-	-	-	-	-	-
58	32	-	-	387	-	-	-	-	-	-
58	34	-	-	18	-	-	-	-	-	-
58	35	-	-	-	-	-	-	13	-	-
58	36	-	-	-	-	-	-	-	-	-
58	43	-	-	-	-	16	-	10	-	-
58	46	-	-	-	-	-	100	-	-	-
58	47	-	-	-	-	10	-	-	-	-
58	48	-	-	-	-	10	-	-	-	-
58	49	-	-	-	-	-	-	-	-	-
58	50	-	-	-	-	3	12	-	-	-
61	1	-	-	323	58	312	614	74	38	-
61	2	10	698	346	2,114	974	529	456	21	-
61	4	-	-	-	1	2	10	11	-	-
61	5	-	-	-	-	-	81	24	-	-
63	1	6	60,190	3,851	8,200	1,497	383	131	-	-
63	2	-	-	177	-	59	43	-	-	-
63	3	1,255	2,780	110	-	170	30	-	176	-
63	4	1,270	16,803	446	91	848	2,445	77	-	38
63	5	28,128	14,405	553	622	2,611	1,375	24	297	-
63	6	282	830	12	-	21	-	13	24	23
63	7	2,452	25,240	-	-	62	-	-	-	-
63	8	436	-	8	-	14	-	-	-	-
63	10	930	1,013	410	-	826	-	-	-	-
63	11	1,628	3,586	18	-	367	92	26	25	-
64	1	9,049	304,782	449	1,600	14	38	-	-	-
64	2	453	1,414	10	161	50	328	40	-	-
64	4	30,630	22,184	150	207	3,078	830	303	216	-
64	5	60	175	103	348	64	1,131	53	-	-
64	8	1,533	8,836	540	4,035	377	1,248	767	324	-
64	9	507	1,928	-	301	19	-	4	-	1
64	10	-	-	-	-	-	50	-	-	-
64	11	-	-	-	-	94	-	-	-	-
64	12	-	-	-	-	-	3	-	-	-
64	14	46	9	17	6	1	6	1	-	-
65	1	1,160	4,170	199	248	921	887	76	76	-
65	2	362	110	76	-	11	64	-	-	-
65	4	34	140	-	-	31	-	4	-	-
71	1	240	800	320	292	580	313	117	7	259
72	1	8,900	60	-	-	24	16	6	-	-
72	2	12,832	10,300	407	411	420	611	452	870	-
72	3	12,501	6,897	647	885	1,942	1,532	311	158	115
72	6	5,000	5,000	444	-	641	-	76	2,400	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
72	7	20	-	-	-	30	-	-	-	-
72	8	40	59	-	-	11	55	40	-	-
72	9	1,881	210	-	-	19	-	-	-	-
72	10	-	-	3	-	15	-	4	-	-
72	11	1,650	1,000	100	-	20	-	-	-	-
72	13	-	-	-	-	9	-	-	-	-
72	16	-	-	-	-	40	-	-	-	-
73	1	976	1,189	-	56	387	389	146	-	12
73	2	2,021	2,549	240	-	75	124	-	-	-
73	3	1,074	12	-	-	64	-	-	-	-
73	7	7,851	33,481	157	131	515	678	216	174	-
73	8	-	2,852	-	-	-	-	-	-	-
73	9	-	-	-	-	-	-	-	34	-
73	10	550	350	3	-	4	3	16	-	-
73	11	1,843	7,304	221	34	433	156	4	-	-
73	12	-	-	-	-	532	-	114	17	-
73	13	-	175	-	-	12	-	-	-	-
73	14	9	-	-	-	4	-	-	-	-
74	1	835	481	-	210	6	-	-	7	-
74	3	-	-	-	-	26	-	-	2,855	-
74	6	197	4,037	20	-	-	-	-	-	-
74	7	-	-	-	-	-	-	-	14	-
74	8	25	-	10	-	35	11	12	-	-
74	11	2,208	1,912	-	-	5,629	131	12	134	-
74	12	4,786	11,356	515	60	1,243	641	163	238	-
74	15	45	-	-	-	-	6	-	-	-
74	18	-	-	476	1,713	2,015	1,663	66	174	-
74	20	4,372	2,259	93	230	115	561	10	16	-
74	25	400	600	-	-	24	30	-	39	5
74	27	200	111	-	50	475	28	-	-	-
74	28	10	8	2	-	16	59	2	-	-
74	29	104,136	231,841	3,959	15,677	13,686	6,369	1,401	1,038	168
74	30	-	-	-	-	-	355	-	-	-
74	31	661	808	-	-	7	-	-	-	971
74	32	225	1,400	-	-	12	-	-	-	-
74	33	556	35	-	-	9	12	-	-	-
74	35	-	-	-	-	16	110	87	-	-
74	36	-	-	-	-	210	-	-	-	-
74	37	17	-	-	-	-	13	-	-	-
74	38	68	4,163	14	-	30	-	-	-	-
74	39	229	506	48	-	10	249	-	8	-
74	40	1,229	273	27	410	18	-	-	-	-
74	42	-	-	154	-	770	-	-	-	-
74	43	-	-	1,706	600	830	-	-	-	-
74	44	-	-	-	-	275	-	-	-	-
74	45	2	5	-	5	2	9	7	-	-
74	46	-	200	-	6	6	-	-	-	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
74	47	1,599	3,180	53	-	54	-	-	-	-
74	48	-	-	-	-	-	-	-	-	-
74	49	1,900	400	630	-	1,925	-	-	-	-
74	50	14,640	11,200	220	280	1,011	454	115	11	-
74	52	-	2,000	-	-	-	-	-	-	-
74	53	-	-	-	-	150	-	-	-	-
74	54	-	-	-	-	-	5	4	-	-
74	55	-	-	-	-	23	-	-	-	-
74	56	-	-	-	-	-	5	-	-	-
74	57	-	-	-	-	-	99	-	-	-
74	58	-	3,800	10	292	10	200	23	-	-
74	59	-	-	-	-	32	-	-	-	-
74	60	70	-	-	-	-	84	-	-	-
74	61	-	348	13	-	69	-	-	-	-
74	63	-	-	-	-	372	-	-	-	-
74	64	-	-	-	-	30	45	-	-	-
81	1	400	3,000	187	-	55	-	-	100	-
81	2	288	2,748	10	33	20	30	400	79	-
81	4	-	760	824	-	-	-	-	-	-
82	1	1,943	2,232	16	-	225	25	1	-	5
82	2	1,525	1,182	120	-	14	-	1	-	-
82	4	3	16	-	-	-	-	-	-	3
83	1	111	-	42	638	41	-	-	-	-
83	2	-	-	-	-	3	-	2	-	-
83	4	-	-	1	-	1	24	6	-	-
84	1	1,078	50,002	796	1,324	1,255	13	30	-	-
84	3	4,710	8,865	225	185	188	2,108	407	-	-
84	5	-	-	-	-	2	21	12	-	-
84	6	23,190	71,504	327	157	2,834	854	265	227	16
84	7	-	-	1,869	825	1,387	15	196	-	-
84	8	40	23	-	-	16	-	-	-	-
84	10	-	628	578	-	14	-	-	-	-
84	11	14	-	-	-	-	-	-	-	-
84	12	3	4	4	2	14	7	6	-	2
84	13	4	2	2	-	2	25	29	-	4
84	14	248	44	-	-	-	-	-	-	-
84	16	768	68	-	-	-	524	-	-	-
84	20	-	-	-	-	12	-	-	-	-
84	21	40	12	-	-	9	-	-	-	-
84	24	-	-	-	-	570	-	-	-	-
84	26	-	-	176	-	1,012	153	-	-	-
84	27	-	-	-	-	-	3	31	-	-
84	29	-	-	-	-	70	-	-	-	-
84	31	-	-	-	-	15	-	-	-	-
84	32	169	3,968	-	-	-	-	-	-	-
84	34	-	-	-	-	3	100	66	-	-
85	1	1,888	2,779	20	682	52	-	-	-	-

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
85	2	6,946	19,499	714	17	304	316	97	226	-
85	3	9,457	2,380	235	685	74	1,379	-	55	-
85	7	400	500	50	-	-	-	-	-	-
85	8	-	-	-	-	5	-	-	-	-
85	9	1,196	96	-	75	25	-	-	675	24
85	11	190	-	-	-	-	-	-	286	-
85	12	560	40	-	-	-	41	-	-	-
86	2	2,045	3,609	19	427	246	148	55	-	-
86	3	19,104	21,198	960	1,406	1,469	640	322	126	-
86	5	40	-	5	-	1	-	4	-	-
86	6	683	-	-	75	-	-	27	33	-
86	7	36	-	39	-	119	95	15	-	-
86	10	-	-	-	-	-	55	2	-	-
86	11	-	-	-	-	-	15	-	-	-
86	13	900	150	150	-	325	-	-	-	-
86	14	-	645	-	-	121	-	-	-	-
86	15	-	-	-	-	-	17	-	-	-
87	2	4,266	12,491	523	270	102	22	-	-	-
87	3	429	735	12	77	36	9	4	4	-
87	5	-	-	-	-	170	-	-	-	-
87	8	12	30	-	-	1	1	-	-	-
87	9	436	17,460	36	-	2,264	-	-	-	-
87	10	537	98	-	-	152	78	-	-	-
88	1	1,253	6,611	273	45	248	1	16	-	-
91	1	20,731	41,219	791	2,807	3,263	1,018	401	1,275	-
91	2	-	-	64	56	904	522	251	40	-
91	3	-	-	-	-	-	206	-	-	-
91	5	634	28,982	44	36	209	183	1	20	-
91	6	20,990	12,569	343	128	170	456	28	-	-
91	7	441	1,054	94	-	112	64	2	5	-
91	9	91	548	-	-	3	-	-	2	-
91	11	67	10,020	10,294	-	128	-	-	-	-
91	13	-	2,700	-	-	-	-	-	-	-
91	15	-	-	-	-	-	-	-	35	-
91	17	-	-	-	-	-	-	-	10	-
91	18	-	-	-	-	-	13	21	-	-
91	19	-	-	-	-	-	8	-	-	-
91	20	1,220	2,000	-	-	151	-	-	-	-
91	23	-	-	-	-	43	-	-	5	-
92	1	8,432	19,033	663	-	514	627	139	1	-
92	2	28	-	-	-	88	-	96	1	-
92	5	5,000	6,000	48	31	473	133	5	25	-
92	6	431	87	10	-	-	9	-	-	-
92	7	159	29	-	-	2	-	-	-	-
92	8	1,080	4,000	6	178	36	-	78	48	-
92	10	1,569	2,429	85	354	82	-	-	2,643	-
92	12	163	-	-	-	-	-	-	-	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
92	13	-	-	-	-	39	-	-	-	-
92	16	-	-	27	-	-	-	-	-	-
92	18	489	-	-	16	2	-	-	-	-
92	20	20	20	-	56	20	-	-	-	-
92	21	-	-	-	-	-	9	-	-	-
93	3	-	-	-	-	-	7	-	-	-
93	4	21,305	83,605	1,483	1,040	5,724	1,820	542	1,040	-
93	6	3,065	10,790	25	43	95	103	54	42	-
93	7	287	2,976	30	237	60	57	5	-	-
93	9	372	1,946	-	-	41	98	-	-	-
93	11	-	-	-	1	182	12	9	-	-
93	13	53	-	-	-	2	-	-	-	-
93	15	1,500	72,000	1,200	-	1,600	-	-	47	-
93	16	4,957	2,328	10	279	1,406	284	19	7	-
93	17	680	2,847	28	5	95	56	8	15	-
93	18	1,089	21,552	7,114	-	2,937	57	11	7	-
93	20	13,483	31,991	383	723	5,998	349	94	192	-
93	25	17	58,800	65	797	150	-	-	18	-
93	26	-	-	286	983	443	16	24	240	-
93	28	2,085	3,667	50	-	244	-	150	58	-
93	29	40,994	80,573	3,662	1,161	1,950	552	545	306	-
93	30	100	1,100	-	-	60	-	-	-	-
93	31	10,400	-	-	-	2,600	-	-	-	-
93	32	1,629	80	6	160	205	963	-	-	-
93	34	24	700	-	-	32	-	-	-	-
93	35	105	6,350	-	-	9	59	-	-	-
93	36	300	16	-	-	362	-	154	-	-
93	37	5	130	-	-	6	15	2	-	8
93	39	5,892	13,906	116	265	587	30	9	43	177
93	40	-	-	-	-	-	28	-	-	-
93	41	40	17,135	35	-	20	-	-	-	-
93	43	21	-	-	-	12	13	-	-	-
93	44	107,920	229,294	5,322	33,791	21,501	6,946	2,956	3,564	151
93	47	30	700	-	-	200	-	-	-	-
93	50	1,452	1,229	40	-	67	67	-	-	-
93	52	450	307	88	-	1,645	-	10	-	-
93	53	10	4	-	2	1	12	8	-	-
93	56	150	275	-	-	396	38	-	13	-
93	58	306	528	124	-	-	-	-	-	-
93	62	1,614	752	-	-	444	-	4	-	-
93	63	-	-	118	-	2,477	-	-	-	-
93	64	-	-	-	-	-	39	380	-	-
93	65	4,550	1,312	1,663	-	1,280	-	-	-	-
93	66	21,686	37,070	807	137	134	14	-	36	-
93	67	2,007	1,961	346	-	2,803	-	56	-	-
93	68	4	700	-	-	4	-	-	-	-
93	71	1,645	13,715	551	-	3,062	-	32	-	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
93	72	40	550	24	-	136	-	-	-	-
93	73	-	-	-	-	28	-	-	-	-
93	74	-	-	-	-	359	-	-	-	-
93	77	57	56	-	-	-	-	-	-	-
93	80	76	-	6	-	-	32	18	-	-
93	81	-	-	-	-	105	-	-	-	-
93	82	12	4	-	-	4	-	-	-	-
93	84	72	30	-	-	12	-	21	-	-
93	86	-	15,000	-	-	275	-	-	-	-
93	88	-	-	-	8	-	-	-	-	-
93	93	-	-	-	-	-	30	-	-	-
93	94	-	-	-	-	100	-	-	-	-
93	95	1,200	1,424	16	-	1,113	-	1	-	-
93	97	186	134	-	10	-	-	-	-	-
93	98	-	-	-	-	175	-	-	-	-
93	99	40	2,000	-	20	120	17	-	-	-
93	100	-	-	-	-	36	-	-	-	-
93	103	250	40	-	-	-	-	-	-	-
93	104	200	300	72	-	6	-	-	-	-
93	105	225	18	-	120	69	-	-	-	-
93	106	-	-	8	-	-	-	-	-	-
93	107	21	42	-	-	-	-	-	-	-
93	108	-	-	-	-	10	-	-	-	-
93	109	-	-	-	-	-	71	29	-	-
93	110	-	-	-	-	-	-	-	1	-
93	115	-	-	-	-	-	-	150	-	-
93	116	6	-	-	-	-	-	-	-	-
93	117	174	282	18	8	4	5	1	-	-
93	118	-	-	-	-	139	-	141	-	-
93	121	-	11,500	179	-	1,179	8	3	-	-
93	122	-	1,491	117	-	-	-	-	-	-
93	123	-	-	-	-	121	-	-	-	-
93	124	75	200	1	4	25	140	291	-	10
93	126	16	-	-	-	-	-	-	-	-
93	127	88	-	-	-	66	-	-	-	-
93	130	3	65	-	-	-	-	-	-	-
93	131	99	348	-	-	4	-	-	-	-
93	133	-	964	-	-	-	-	-	-	-
93	134	-	-	-	-	-	-	-	12	-
93	136	-	-	-	-	-	2	3	-	-
93	138	40	24	-	-	-	-	-	-	-
93	140	-	-	-	-	77	-	-	-	-
93	141	-	250	-	-	58	-	-	-	-
93	144	-	-	1	-	6	-	-	-	-
93	145	-	-	208	-	2,702	-	-	-	-
93	147	-	260	-	-	462	-	-	-	-
93	151	2,200	500	-	-	6	-	-	20	-

USDA/APHIS

FY 1982

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
93	152	-	-	-	-	-	2	2	-	-
93	153	-	-	-	-	-	-	-	10	-
93	155	-	2,427	1,329	-	7,440	-	-	-	-
93	156	-	-	-	-	-	94	35	-	-
93	157	-	-	4	-	8	-	-	-	-
93	158	-	41	-	-	412	-	-	-	-
93	159	708	-	-	-	609	-	-	-	-
93	160	5	-	-	-	-	-	-	-	-
93	161	2	3	-	-	26	-	-	-	-
93	165	25	802	-	-	36	-	-	-	-
93	166	-	-	-	-	1	10	-	-	-
93	169	1,952	-	-	-	511	-	-	-	-
93	171	-	-	-	-	1	13	-	-	-
93	172	-	-	-	-	-	-	-	2	-
93	173	-	-	-	-	55	-	-	-	-
93	174	-	-	-	-	61	-	-	-	-
93	176	-	-	-	-	13	-	-	-	-
93	178	-	-	-	-	-	-	13	-	-
93	179	-	-	-	-	-	-	1	1	-
93	180	-	-	-	-	3	-	-	-	-
93	181	-	238	98	-	-	-	-	-	-
93	183	-	-	454	-	-	-	-	-	-
93	184	-	-	-	-	176	-	-	-	-
93	186	-	-	-	-	-	-	12	-	-
94	1	-	-	120	75	138	340	52	1,903	-
94	2	-	-	-	-	26	-	-	-	-
94	4	-	-	-	-	-	-	-	36	-
94	5	-	-	-	-	110	-	-	-	-
94	8	-	-	-	-	5,000	-	-	-	-
94	10	-	-	4	-	-	2	9	4	-
94	11	-	-	-	-	-	-	-	1	-
95	2	-	-	258	2,298	456	37	37	105	-
95	3	600	75	-	-	127	20	-	35	-
96	1	-	-	-	-	-	3	-	-	-
FED	1	160,539	387,916	19,842	14,094	9,463	770	495	3,928	-
FED	2	57,511	198,050	16,277	5,655	3,482	2,354	674	922	742
FED	3	103,520	187,250	4,134	7,069	13,888	8,360	2,110	431	408
FED	4	20,140	45,428	1,641	779	1,573	1,066	224	993	71
FED	5	4,937	8,107	817	884	699	66	12	-	24
FED	6	12,832	132,112	5,342	11,009	2,449	80	26	13	3,480

USDA/APHIS

FY 1983

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
DC	1	35	-	-	-	99	108	-	-	-
DC	3	217	517	8	-	28	47	19	-	-
DC	4	7,548	17,506	77	42	226	299	779	10	8
DC	5	6,100	9.407	102	266	228	234	8	18	-
DC	6	-	-	7	-	-	-	-	-	-
DC	7	170	42	-	-	19	-	-	-	67
DC	9	4,744	1,810	275	377	103	119	144	3	-
11	1	-	-	-	-	44	-	-	-	-
11	2	84	85	-	-	42	-	6	-	-
11	3	64	100	-	-	6	-	-	-	-
11	6	670	695	15	40	56	-	-	-	150
11	8	300	450	-	-	2	-	-	-	-
11	9	42	4	-	176	-	-	-	-	-
11	10	-	-	-	-	30	-	-	-	-
11	11	-	-	3	-	59	-	-	-	-
11	13	-	-	-	-	153	-	-	-	-
11	14	-	146	-	-	-	-	-	-	-
12	1	273	27	-	5	4	-	-	-	-
12	2	24	-	-	-	-	-	-	-	-
12	3	1,445	1,440	10	134	28	-	-	-	-
13	1	8,275	13,633	343	241	1,746	36	86	-	-
13	5	2	-	4	52	-	-	-	-	-
13	9	-	-	-	-	-	4	6	-	-
14	3	842	34,315	-	-	169	-	-	-	-
14	4	72	30	-	-	-	-	-	-	-
14	5	-	-	-	46	6	-	-	-	-
14	6	-	-	102	1,436	668	17	-	88	-
14	7	122	79	13	-	7	3	3	-	-
14	8	-	-	-	-	38	-	-	-	-
14	10	564	2.260	52	-	1,534	-	-	34	-
14	11	-	-	98	-	-	-	-	-	-
14	12	3,656	4,052	-	5,725	63	-	-	40	-
14	13	1,200	4,000	18	-	120	70	2	-	-
14	14	20.517	23.703	756	150	2,839	2,009	383	32	-
14	15	8,675	1,995	1,410	116	1,017	346	20	10	-
14	17	-	-	469	172	2,990	164	36	231	-
14	18	-	-	200	200	50	19	100	-	-
14	19	20.669	151,827	2,000	3,956	1,393	1,721	250	361	-
14	20	6.537	11,742	242	136	568	24	15	4	-
14	21	1,802	8,873	67	-	150	-	-	10	32
14	22	-	-	125	725	268	12	230	16	-
14	24	-	25	-	16	8	-	-	-	-
14	25	1	3	1	2	1	4	5	-	-
14	30	320	11,760	30	55	131	-	-	45	-
14	32	2,634	1,839	230	182	235	-	-	-	-
14	33	2,038	9.630	71	45	175	-	-	-	-
14	35	33	880	506	1,537	1,793	598	84	41	-

USDA/APHIS

FY 1983

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
14	37	555	250	-	175	26	-	-	-	-
14	38	260	2,426	-	6	80	-	-	-	-
14	44	5,384	2,683	542	-	203	-	-	-	-
14	45	-	-	-	-	-	22	-	-	-
14	50	-	-	-	1,251	20	-	-	-	-
14	53	-	1,350	452	-	69	-	-	-	-
14	54	-	-	-	1,430	-	-	-	-	-
14	61	-	75	-	-	6	-	-	-	-
14	68	165	550	-	-	-	-	-	-	-
14	69	-	-	12	-	120	-	-	-	-
14	73	-	-	2	-	2	355	300	-	-
14	74	155	70	5	10	24	50	15	-	-
14	78	258	426	89	-	10	-	-	-	-
14	80	-	-	392	165	1,549	-	-	-	-
14	81	-	6,500	-	1,400	-	-	-	30	-
14	83	9,890	5,779	288	16	1,974	144	1	-	-
14	84	1,669	2,312	-	328	477	-	-	31	-
14	86	150	38	23	-	260	-	-	-	-
14	88	120	20	-	40	-	-	-	-	-
14	89	126	113	-	-	192	-	-	-	-
14	92	250	-	603	77	450	-	-	-	-
14	93	-	-	-	1,356	-	-	-	-	-
14	95	-	-	17	-	-	-	-	-	-
14	96	-	-	1	-	77	-	-	23	-
14	97	-	-	-	-	-	32	-	-	-
14	98	-	-	-	-	-	19	-	-	-
14	99	-	-	-	-	12	-	-	-	-
14	100	-	3,960	-	-	-	-	-	-	-
14	101	50	6,000	8	-	650	-	-	-	-
14	102	-	-	109	-	646	23	-	-	-
14	105	402	148	6	-	20	16	-	12	-
15	2	-	-	544	-	153	104	415	-	-
15	3	-	-	-	60	103	54	116	81	-
15	4	1,428	1,812	80	4	142	-	-	9	-
15	5	856	-	2	-	-	60	-	-	-
15	6	-	-	-	30	8	14	-	-	-
15	10	-	60	6	-	6	-	-	-	-
15	14	-	-	-	-	19	-	-	-	-
16	1	4,512	21,650	2,953	2,134	3,202	723	300	178	-
16	2	551	-	-	-	-	-	-	-	-
16	4	316	-	259	-	66	-	-	30	-
16	7	-	-	-	-	58	112	-	-	-
16	8	-	-	75	12	308	152	72	8	-
16	11	-	-	-	50	4	-	-	-	-
16	16	-	-	-	-	6	-	-	-	-
16	23	55	-	-	-	12	-	-	-	-
16	28	-	-	-	-	-	739	-	-	-

USDA/APHIS

FY 1983

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
16	29	-	-	4,596	3	35	16	19	16	-
16	32	-	-	-	-	374	190	-	16	-
16	33	-	-	149	-	-	-	-	-	-
21	1	75	30	-	-	55	50	-	-	-
21	2	477	213	-	856	-	-	-	-	-
21	3	-	-	2,371	-	717	733	180	-	-
21	7	114	12	148	47	21	-	-	-	-
21	8	121	2,131	474	-	865	-	-	-	-
21	11	-	-	13,489	-	1,344	2,020	270	815	-
21	12	-	-	134	8	28	-	-	-	-
21	14	-	-	6	644	681	-	-	51	-
21	15	3,160	-	-	172	95	10	-	6	-
21	17	67	210	55	-	20	101	38	-	-
21	22	-	-	1,117	-	347	245	-	-	-
21	24	-	-	19,078	-	2,332	389	28	1,144	-
21	28	396	-	-	-	-	27	150	-	-
21	33	143	326	-	439	201	88	40	-	-
21	34	-	-	341	237	234	-	-	-	-
21	36	-	-	73	453	723	173	392	34	-
21	38	-	-	-	-	182	5	-	-	-
21	40	-	-	382	1,111	673	263	30	33	-
21	42	-	-	-	-	-	12	6	-	-
21	43	-	-	2,000	-	1,650	-	-	-	-
21	45	240	210	150	-	120	-	-	-	-
21	46	150	-	-	-	2	-	-	-	-
21	48	-	-	1,176	535	555	435	30	110	-
21	49	-	-	-	-	4	36	-	-	-
21	50	-	-	3,057	-	1,547	-	-	-	-
21	59	-	-	-	-	195	78	-	-	-
21	60	3,600	3,500	590	-	997	324	25	-	-
21	61	-	-	-	-	8	-	-	-	-
21	62	620	-	-	-	20	-	-	33	-
21	63	50	75	-	-	-	-	-	-	-
21	64	15	460	-	-	13	-	-	-	-
21	66	-	-	-	-	-	18	-	-	-
21	67	15	-	-	-	2	-	-	-	-
21	69	327	464	3	-	249	237	-	-	-
21	70	-	-	116	-	24	-	-	-	-
21	71	9,300	250	2,000	100	570	200	-	6	-
21	72	-	-	71	301	233	52	108	4	-
21	73	1,354	78	21	30	14	122	2	-	-
21	74	3,739	385	143	-	57	74	24	5	-
21	76	210	40	35	-	4	42	-	-	-
21	79	410	-	-	-	-	-	-	8	-
21	81	-	-	72	15	258	341	9	-	-
21	86	50	100	-	-	9	-	-	-	-
21	87	10,099	402	3,024	240	297	28	-	-	-

USDA/APHIS

FY 1983

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
21	88	-	54	6,794	613	1,120	461	19	23	-
21	89	-	-	27	-	-	-	70	-	-
21	91	162	28	4	-	-	-	-	-	-
21	96	6	228	-	103	15	-	-	-	-
21	98	68	38	-	-	12	-	-	-	-
21	100	25	-	-	-	-	-	-	-	-
21	103	-	-	8	80	455	174	793	3	-
21	104	16,304	6,755	433	587	1,006	1,160	227	219	-
21	107	2,907	140	4	262	15	80	-	-	-
21	109	22,025	19,381	271	441	595	836	127	96	-
21	110	-	-	8	264	86	-	-	-	-
21	112	-	-	-	-	2	42	-	-	-
21	114	35	-	805	551	1,282	338	326	3	-
21	115	-	-	-	-	30	110	-	-	-
21	116	1,290	2,652	10	-	138	42	-	-	-
21	117	10	-	-	1	2	133	12	-	-
21	118	10,221	26,167	880	1,529	448	310	197	440	28
21	119	-	-	181	419	810	326	101	10	-
21	122	237	66	2	-	20	-	-	-	-
21	123	428	-	-	-	44	69	-	-	-
21	124	15,894	313,991	1,595	538	715	436	6	125	-
21	126	238	170	-	-	-	-	-	-	-
21	127	-	-	873	36	1,602	48	52	-	-
21	128	448	1,430	4	-	87	-	-	-	-
21	129	80	-	-	-	60	170	-	-	-
21	130	700	2,600	-	40	8	149	5	-	-
21	131	6,628	11,904	-	-	1,998	147	44	-	-
21	132	-	-	750	263	569	367	342	27	-
21	133	13,434	22,034	956	474	392	-	66	40	-
21	134	-	-	-	-	-	-	-	4	-
21	135	-	450	150	250	26	-	-	-	-
21	136	18,113	27,680	1,248	1,443	2,865	1,378	1,168	478	32
21	137	1,430	104	-	-	95	133	3	-	-
21	138	2,300	2,350	-	-	43	26	45	-	-
21	139	4,054	1,910	-	-	299	82	-	8	-
21	140	300	50	50	-	60	25	10	-	-
21	141	62	-	-	-	-	-	-	-	-
21	142	3,171	1,174	9	174	36	4	4	15	-
21	146	-	268	34	467	8	-	-	-	-
21	148	363	897	-	-	13	-	-	-	-
21	151	-	-	4	-	13	-	-	-	-
21	153	-	-	396	-	394	2	-	-	-
21	154	-	-	1,650	-	-	-	-	-	-
21	159	-	-	-	-	150	-	-	-	-
21	162	-	-	-	-	-	-	-	78	-
21	164	1,895	170	590	110	447	25	-	-	-
21	165	-	-	88	-	157	-	-	-	-

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
21	166	179	760	-	-	99	-	-	-	-
21	167	768	930	-	-	84	-	-	-	-
21	168	-	8,628	-	-	198	-	-	-	-
21	170	123	20	-	-	8	-	-	-	-
21	171	-	-	31	-	-	-	-	-	-
21	172	-	-	-	-	30	-	-	-	-
21	173	-	-	739	97	3,865	191	-	-	-
22	4	-	-	53	-	900	-	10	-	-
22	5	-	7,840	11	-	27	-	-	39	20
22	6	-	-	7,528	2,090	2,551	2,072	2	30	-
22	9	31,770	3,649	901	165	898	716	-	721	-
22	10	10,500	87,088	-	-	4,548	164	-	-	-
22	12	13,907	454	199	-	760	423	-	-	-
22	14	-	-	3,080	-	2,296	1,502	32	54	-
22	16	4,415	4,227	5,890	385	748	83	-	-	-
22	20	8,720	9,698	279	641	287	494	178	14	-
22	21	-	-	186	-	637	66	-	-	-
22	22	2,706	3,556	-	967	112	-	94	25	-
22	25	4,328	6,301	1,391	925	236	14	6	21	-
22	26	-	200	12	-	579	-	-	-	-
22	28	30,987	146,546	3,106	129	1,371	761	5	38	-
22	29	4,000	200	25	-	300	37	-	-	-
22	30	-	-	8,589	2,265	6,645	1,759	384	691	-
22	31	-	-	-	-	-	75	1	-	-
22	32	-	-	9,807	1,403	1,412	738	118	69	-
22	33	-	-	4,058	5	4,781	381	-	195	-
22	36	4,849	3,735	11,671	1,290	1,541	1,351	51	259	-
22	37	15	1,930	-	-	2	-	-	-	-
22	39	286	50	196	-	-	-	-	-	-
22	41	25	17,245	-	-	1,808	18	-	-	-
22	42	-	-	209	-	236	352	-	3	-
22	44	-	-	471	8,210	82	476	133	-	-
22	45	-	-	-	-	16	-	-	-	500
22	50	1,630	145	246	-	94	-	-	-	-
22	55	-	57	35	2,586	36	4	1	-	-
22	63	-	-	14	-	389	-	-	-	-
22	64	-	-	62	-	-	-	-	-	-
22	65	35	7,354	-	-	-	-	-	-	30
22	67	185	125	4	-	8	5	-	-	-
22	69	5,110	305	1,375	9	2,810	-	-	-	-
22	71	-	-	2,469	-	696	-	-	-	-
22	72	126	370	-	-	17	-	-	-	-
22	73	903	86	40	-	699	-	-	-	-
22	74	3,310	620	1,169	-	1,164	-	-	-	-
22	75	625	-	618	-	275	-	-	-	-
22	76	160	20	-	2	4	14	4	-	-
22	77	-	-	-	-	232	-	-	-	-

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
22	78	-	150	50	-	50	-	-	-	-
22	80	-	-	552	-	-	920	61	-	-
22	82	1,500	800	200	30	800	-	-	-	-
22	86	-	-	-	-	-	115	46	-	-
22	87	624	8,026	1,445	-	3,714	9	-	-	-
22	88	-	-	384	-	1,330	-	-	-	-
22	89	9,608	477	-	-	-	6	39	-	-
22	90	250	275	10	3	1	-	-	-	-
22	91	-	-	19	-	24	-	-	-	-
22	92	-	410	-	-	-	-	-	-	-
22	93	240	24	595	-	-	-	-	-	-
23	1	2,482	6,386	175	150	38	-	8	-	-
23	3	-	-	-	-	67	191	-	-	-
23	5	4,390	6,536	-	-	559	46	206	-	-
23	6	-	-	-	-	-	100	-	-	-
23	8	-	-	-	-	50	-	-	-	-
23	9	21,712	4,630	1,422	-	50	400	-	10	-
23	10	626	48	-	-	34	-	81	-	2
23	11	8,312	27,140	588	390	1,020	157	657	70	-
23	12	-	-	7,053	18,770	2,237	1,667	450	12	-
23	13	253	47	4	-	286	-	-	-	-
23	15	59,595	164,332	8,174	33	706	1,067	-	85	-
23	16	34,068	17,606	1,156	15	930	703	256	316	123
23	18	-	146,012	8,857	344	2,746	1,055	161	20	-
23	19	-	-	93	-	112	335	13	-	-
23	21	32,733	15,599	451	451	1,593	213	137	196	950
23	22	1,216	11,706	-	-	87	29	4	-	-
23	23	23,855	25,285	1,488	3,521	3,120	1,184	1,087	8	361
23	24	14,957	8,970	380	-	3,398	-	-	-	-
23	25	196	365	-	5	11	76	3	-	-
23	26	30	-	4	-	31	13	4	-	-
23	27	10,562	4,980	712	352	443	359	654	-	20
23	29	256	1,374	-	60	120	-	5	-	-
23	31	6,349	19,452	306	1,758	104	234	22	340	-
23	32	34	20	6	-	182	310	16	-	-
23	33	4,223	17,921	214	-	78	51	185	-	146
23	35	15,392	42,260	290	-	338	305	-	18	-
23	36	-	-	-	-	4	-	-	-	-
23	37	6,258	3,107	325	-	1,918	100	-	-	-
23	41	-	-	-	-	-	7	-	-	-
23	45	3,449	5,659	-	-	-	28	-	-	-
23	46	850	350	-	280	12	-	-	50	-
23	48	1,200	-	-	-	-	-	5	-	-
23	49	31	651	-	-	354	-	-	-	-
23	50	144	106	-	-	-	-	-	-	-
23	54	-	4,650	-	-	-	-	-	-	-
23	55	3,036	59,324	56	105	463	-	-	-	-

USDA/APHIS

FY 1983

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
23	57	6	430	-	-	10	-	-	-	-
23	58	65	-	21	-	55	-	-	-	-
23	60	-	20	50	-	11	-	-	-	-
23	61	3,819	100	549	-	4,097	-	-	-	-
23	63	40	-	-	-	-	-	-	-	-
23	64	35	900	-	-	3	-	-	-	-
23	65	-	-	-	-	-	346	43	-	-
23	67	25,180	8,600	-	-	102	1,717	76	523	-
23	69	600	875	56	-	48	25	-	-	-
23	75	9	5	2	3	-	16	9	-	-
23	76	138	290	-	60	15	-	-	-	-
23	78	600	1,000	60	500	15	11	12	-	-
23	79	-	-	-	-	-	-	12	-	-
23	82	6,388	161	1,337	-	2,707	-	-	-	-
23	84	-	28,790	1,992	-	706	-	-	-	-
23	85	551	3,692	12	35	30	50	4	-	-
23	87	-	129	-	-	282	-	-	-	-
23	88	2,468	1,350	514	-	-	-	-	208	100
23	90	40	80	-	35	-	6	-	20	-
23	91	318	1,268	12	-	247	-	-	87	-
23	92	3,696	3,860	137	660	132	117	-	19	1
23	94	200	24	-	-	-	1,115	-	-	-
23	95	-	-	-	-	6	-	-	-	-
23	96	-	200	20	-	95	-	-	-	-
23	97	626	-	-	-	500	-	-	-	-
23	99	3,570	-	-	-	1,149	-	-	-	-
23	100	49	-	31	-	11	-	-	-	-
23	101	-	-	-	-	-	142	12	-	-
23	102	142	16	-	-	14	-	-	-	-
23	103	872	312	-	-	7	-	-	-	-
23	106	-	2,880	-	-	898	-	-	-	-
23	107	6,450	2,216	1,612	-	1,967	-	-	-	-
23	108	-	-	458	25	-	2	-	-	-
23	109	3,062	2,243	286	-	273	-	-	-	64
23	110	-	-	-	-	15	-	-	-	-
23	111	55	-	-	-	-	-	-	4	-
23	112	-	-	-	-	-	11	-	-	-
23	113	-	24	-	-	1	-	-	-	-
23	114	-	-	-	-	106	-	-	-	-
31	1	237	-	14	-	-	151	10	-	-
31	2	250	362	-	-	-	-	7	-	-
31	6	530	885	10	24	20	-	-	-	-
31	9	-	-	5,190	-	1,265	-	-	-	-
31	10	-	-	6,125	221	1,275	236	-	-	-
31	11	3,855	4,456	102	-	102	291	25	32	32
31	13	-	-	51	-	224	51	26	-	-
31	14	-	33	315	68	964	1,669	217	136	-

FY 1983

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
31	16	3,432	1,610	2,835	-	1,066	49	-	-	-
31	17	-	-	121	-	360	680	139	-	-
31	19	110	325	16	11	-	6	-	-	-
31	21	3,160	197,046	583	45	93	117	30	33	-
31	25	584	368	6	9	13	336	3	-	-
31	26	750	845	58	-	7	-	-	-	-
31	27	-	-	2,215	777	539	1,380	502	30	-
31	28	13,674	15,857	354	683	1,241	892	389	29	1,613
31	29	300	300	21	-	54	-	-	-	-
31	30	4,016	1,018	51	-	83	12	-	9	-
31	31	22	55	-	-	55	197	-	-	-
31	33	15	-	25	-	-	195	-	-	-
31	36	-	1,500	-	-	1	-	9	-	-
31	40	1,469	735	15	-	33	10	-	-	-
31	42	200	200	-	-	8	-	10	-	-
31	43	11,087	20,120	368	244	346	485	77	8	-
31	47	-	-	-	-	74	-	-	-	-
31	48	-	9,668	694	-	1	-	-	-	-
31	49	133	121	-	-	2	-	-	9	-
31	50	91	40	-	-	2	-	-	-	-
31	53	28	10	1	8	14	4	6	5	-
31	54	70	-	-	-	19	-	-	12	-
31	55	4,374	7,393	4	237	142	194	59	-	-
31	57	-	-	-	-	-	126	-	-	-
31	58	174	2,800	29	3	20	-	-	-	-
31	59	-	-	797	-	-	-	-	-	-
31	61	26	30	-	-	-	25	3	-	-
31	62	-	-	-	-	-	25	-	-	-
31	63	-	-	12	-	18	17	-	-	-
31	65	-	-	-	-	3	30	-	-	-
31	66	60	38	-	-	-	-	158	-	-
31	68	1,100	23,964	593	11	4,915	16	-	-	-
31	70	-	500	-	1	-	-	-	-	-
31	71	138	-	-	-	-	-	-	-	-
31	73	-	-	-	-	10	-	-	-	-
31	78	140	-	-	-	-	-	-	-	-
31	79	433	-	-	121	18	-	-	-	-
31	80	36	6	40	14	-	39	2	-	-
31	81	60	48	-	-	12	-	-	-	-
31	82	2,477	7,880	16	-	292	15	138	-	88
31	84	26,158	85,628	1,717	40	421	1,010	348	74	-
31	86	-	810	3	-	4	-	-	-	-
31	87	151	43	193	-	69	33	138	-	-
31	88	12	9	2	-	2	14	7	-	-
31	89	-	-	989	-	2,750	229	-	-	-
31	90	4,821	28,796	183	-	99	59	12	-	-
31	91	1,110	22,040	75	-	-	-	-	-	-

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
31	92	2,435	970	6	608	132	540	80	7	-
31	93	-	53	13	-	40	-	-	-	-
31	94	-	-	-	-	5	-	-	-	-
31	96	-	-	-	-	40	-	-	-	-
32	1	-	-	3,027	32	3,613	276	90	1,806	-
32	2	-	-	-	-	-	15	-	-	-
32	3	-	-	12,108	160	4,530	2,165	2,029	100	-
32	4	20	10	-	-	-	-	-	-	-
32	5	-	-	1,228	-	491	646	13	62	-
32	6	37,565	16,830	1,470	821	1,076	2,600	666	80	628
32	7	9,777	56,588	291	447	313	657	18	-	-
32	9	-	-	7	-	68	-	-	-	-
32	10	300	900	-	-	4	2	-	-	-
32	11	-	-	2	230	7	-	-	-	-
32	14	185	43	2	-	2	-	-	-	-
32	16	470	1,250	-	121	6	3	22	-	-
32	17	140	10	-	-	-	-	-	-	-
32	20	7,982	110	156	108	753	-	-	-	-
32	21	-	-	-	-	60	-	-	-	-
32	23	-	-	75	-	165	6	-	-	-
33	3	-	-	-	100	60	-	-	-	-
33	6	326	336	-	-	393	24	8	-	-
33	8	5,821	198,674	531	5,632	530	-	-	-	1,000
33	9	3,549	8,358	84	381	55	189	27	42	226
33	10	1,000	4,500	-	62	9	29	-	-	-
33	11	-	100	-	-	3	-	-	-	-
33	13	899	1,640	2	25	22	110	-	5	-
33	14	6,690	7,699	422	19	424	169	87	39	-
33	18	1,769	166	677	939	2,907	883	450	656	-
33	20	4,093	14,873	12	310	128	507	-	-	24
33	22	-	-	801	47	809	318	5	678	-
33	23	3,026	13,340	7,968	-	8,276	270	-	-	-
33	24	10,644	7,829	823	45	550	1,398	257	26	-
33	25	-	-	922	6	7,089	573	-	64	-
33	26	691	53	4	189	23	22	-	-	-
33	27	1,660	3,804	59	26	111	210	-	-	-
33	28	68,213	92,730	6,564	536	781	1,176	205	276	-
33	29	16,760	24,403	271	3,499	840	867	273	51	-
33	30	30,366	19,828	436	221	1,618	1,452	235	390	-
33	31	-	-	-	-	-	433	-	-	-
33	33	41,740	2,825	4,082	-	8,220	10	-	-	-
33	34	-	-	1,276	-	1,522	1,246	173	-	-
33	35	800	4,000	30	25	420	210	33	5	-
33	36	-	-	-	-	35	-	-	5	-
33	38	-	-	-	-	12	28	2	-	-
33	40	21,241	29,026	276	2,870	1,147	656	424	49	-
33	41	-	-	-	-	-	50	-	-	-

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
33	43	226	690	-	-	924	-	-	-	-
33	44	1,659	181	-	-	6	-	-	-	-
33	45	120	-	-	50	12	-	-	-	-
33	47	2,000	-	-	-	6	12	6	-	-
33	48	-	-	-	-	-	6	-	-	-
33	49	80	100	-	-	4	-	-	-	-
33	51	50	12	3	14	-	-	-	-	-
33	53	60	46	-	-	-	-	-	-	20
33	56	444	-	-	-	-	-	-	-	-
33	58	-	-	-	-	24	-	-	-	-
33	59	120	100	-	-	2	-	-	-	-
33	62	350	200	-	-	14	-	-	-	-
33	67	4,944	1,302	29	-	-	-	-	-	2,000
33	68	499	92	-	-	56	18	-	-	-
33	69	1,000	220	52	-	-	-	-	7	-
33	72	-	200	-	-	-	-	-	-	-
33	75	-	-	-	-	328	181	45	-	-
33	79	1,280	-	-	-	7	-	-	-	-
33	81	-	-	-	-	-	195	278	-	-
33	83	20	36	-	-	9	-	-	-	-
33	84	3	8,242	-	-	-	-	-	-	-
33	87	-	-	-	-	63	-	-	-	-
33	88	12	18	-	-	1	-	-	-	-
33	89	-	57	-	-	-	-	-	-	-
33	90	-	-	50	-	1,500	-	-	-	-
33	91	8,452	2,009	414	-	1,348	44	-	-	-
33	94	163	403	66	-	620	-	9	-	-
34	1	42,831	42,796	1,617	1,282	3,235	2,028	487	309	-
34	3	40,650	17,642	296	8	5,028	1,666	32	333	-
34	5	52,155	177,352	11,741	-	3,202	1,297	8	146	-
34	6	-	-	13	-	20	26	11	-	-
34	7	15	71	-	-	-	34	-	-	-
34	8	35,145	75,660	8,418	-	2,151	423	-	348	-
34	9	68	-	-	-	32	6	-	-	-
34	10	7,105	4,890	12	-	274	344	38	20	-
34	11	2,817	4,953	-	-	-	4	-	3	-
34	12	-	2,000	-	-	-	520	-	-	-
34	14	-	-	170	799	666	887	64	39	-
34	15	87,937	285,510	7.675	9.455	4,578	789	508	237	-
34	16	-	-	-	-	-	26	-	-	-
34	17	-	-	1,133	522	380	1,045	467	152	-
34	18	36	212	-	-	54	48	-	-	-
34	19	275	-	-	-	-	-	-	-	-
34	20	10	-	-	100	-	-	-	-	-
34	21	94	2.510	-	-	-	-	-	-	-
34	24	100	1,050	28	190	5	-	-	-	-
34	27	-	66,493	6,917	1,836	624	-	-	21	-

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
34	28	-	-	-	-	-	26	-	-	-
34	32	1,084	1,960	890	-	1,020	4	-	-	-
34	33	620	1,030	-	55	10	-	-	-	155
34	34	-	-	-	-	16	192	40	-	-
34	35	288	6	164	40	-	-	-	-	-
34	36	21	251	18	-	20	28	21	-	-
34	37	130	512	12	80	35	56	8	-	-
34	38	2,417	450	-	54	952	91	2	-	-
34	39	1,000	5,400	15	-	-	-	-	-	-
34	42	5	3,883	-	-	-	-	-	-	-
34	43	-	-	-	-	21	-	-	-	-
34	44	315	50	-	-	6	-	-	-	-
34	47	225	350	-	-	3	1	-	-	-
34	50	-	-	-	-	-	-	-	6	-
34	52	2,045	915	95	22	83	30	-	-	-
34	54	3,560	3,252	1,705	-	699	175	-	-	-
34	59	-	150	-	-	-	-	-	-	-
35	1	-	1,000	2,937	4,147	1,508	1,058	744	1,600	3,167
35	8	54	48	-	-	24	-	-	-	-
35	9	-	-	-	25	373	59	-	-	-
35	11	1,611	5,853	129	168	104	-	-	-	-
35	12	-	15,720	168	4,610	441	366	116	-	-
35	16	32	140	6	14	6	83	78	-	-
35	20	2	60	62	-	-	-	-	-	-
35	21	30	24	-	16	8	-	-	-	-
35	23	-	-	-	-	2	10	6	-	-
35	24	3	64	6	-	58	-	-	-	-
35	27	82	27	8	-	-	-	-	-	-
35	29	7,201	10,989	359	-	2,166	1,370	251	41	-
35	30	-	-	1,143	-	3,097	342	1	108	-
41	3	350	622	130	-	3	159	39	-	-
41	5	19,421	67,525	1,421	757	2,527	2,715	1,054	89	-
41	6	5,611	44,734	498	312	1,241	1,805	369	57	-
41	7	1,056	-	15	-	20	178	4	-	-
41	8	5,240	11,430	6,468	60	2,188	84	64	2	-
41	11	158	6	-	-	12	-	-	-	-
41	12	20	30	-	-	-	-	-	-	-
41	14	480	267	-	-	-	-	-	-	-
41	15	350	400	12	-	250	300	-	-	-
41	18	-	200	175	-	6	-	-	-	-
41	19	-	-	-	-	329	-	-	-	-
41	21	-	-	-	-	-	65	-	-	-
41	22	11	-	-	-	-	266	-	-	-
41	23	-	-	-	-	-	10	-	-	-
41	24	-	-	-	-	75	-	-	-	-
41	25	3	168	19	-	87	-	-	-	-
41	26	-	47	-	-	6	-	-	-	-

USDA/APHIS

FY 1983

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
41	27	-	1,762	-	-	-	-	-	-	-
42	1	-	-	2,304	3,344	627	-	-	-	-
42	2	85	100	-	-	1	294	4	-	-
42	3	3,180	17,200	810	-	908	777	242	-	-
42	4	-	-	589	608	5,287	2,050	1,162	161	-
42	5	162	-	-	-	15	23	-	-	20
42	9	-	16,266	519	400	104	58	43	-	-
42	11	140	-	-	-	36	-	-	-	-
42	12	-	-	1,062	-	158	-	-	-	-
42	15	-	1,200	-	-	15	-	-	-	-
43	1	310	60	-	-	124	39	28	-	-
43	2	1,062	2,190	141	-	1,148	366	-	-	-
43	3	96	120	-	-	27	-	-	-	-
43	5	1,567	6,798	206	-	84	112	-	-	-
43	6	1,043	598	6	-	1,648	1,828	1,269	-	-
43	7	3,024	21,597	-	135	117	221	-	-	100
43	8	81,714	63,858	3,120	1,478	10,214	2,670	1,162	318	-
43	9	2,628	902	22	-	57	-	-	5	-
43	10	13,027	27,486	1,764	966	960	641	325	394	-
43	11	10,702	12,436	116	32	878	514	107	28	-
43	12	-	-	14	-	46	416	95	-	-
43	14	17	53,322	3,876	7,565	954	392	200	-	-
43	15	93	340	-	-	311	-	-	-	-
43	21	9	-	-	-	26	-	-	-	-
43	22	12	-	-	-	-	-	-	-	-
43	23	-	-	-	-	81	-	-	-	-
43	24	3,349	619	154	-	51	68	23	-	-
43	25	-	-	-	-	25	-	-	-	-
43	26	1,500	-	-	-	825	-	-	-	-
43	28	-	2,000	-	-	-	-	-	-	-
43	29	2	2	5	3	1	33	15	-	-
43	30	21	18	8	-	14	140	47	-	-
43	31	74	18	-	-	24	-	-	-	-
43	33	17,612	376	6	-	623	24	-	-	-
43	35	-	-	-	-	-	13	-	-	-
43	36	-	-	-	-	154	-	-	-	-
43	999	-	1,040	-	-	2,900	-	-	-	-
45	1	2,516	4,621	61	-	456	92	98	-	-
45	2	5,550	2,700	110	4	59	75	16	-	30
45	3	20	-	-	-	-	-	-	-	-
46	1	573	125	36	650	55	72	2	-	25
46	2	1,704	3,200	50	-	128	22	-	-	-
47	2	457	8,229	170	76	315	404	18	-	-
47	4	12,757	15,201	59	2,912	1,303	296	39	40	482
47	5	-	1,500	175	1,125	150	88	50	-	-
47	7	40	120	-	-	5	-	-	-	-
47	9	158	-	-	-	6	-	32	-	-

USDA/APHIS

FY 1983

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
47	10	-	19,814	239	980	430	12	8	-	-
47	13	-	-	5	3	3	9	3	-	-
48	1	-	1,300	96	178	216	524	46	5	-
48	2	10,500	12,000	63	-	630	31	5	-	-
48	3	6,517	24,903	58	3,771	1,317	327	109	10	-
48	4	4,976	2,107	125	-	291	72	-		-
48	5	718	92,367	5,135	5,060	2,597	159	85	-	-
48	9	1	3	-	-	-	-	3	11	-
48	11	-	18,212	116	-	116	200	74	-	-
48	14	100	327	-	-	8	-	-	-	-
48	15	130	100	19	-	34	-	-	-	-
48	17	17	50	-	-	12	-	-	-	-
48	18	14	60	-	-	12	-	-	-	-
48	19	-	16,985	220	4,900	84	51	73	-	-
48	20	80	50	24	-	-	-	-	-	-
48	21	10	7	10	-	-	-	-	-	-
50	1	2,374	167	-	-	194	9	-	-	-
50	2	293	622	10	66	9	-	-	-	-
50	3	27,772	8,257	152	-	466	112	-	-	-
50	4	68,491	98,974	7,560	15,000	770	422	45	14	-
50	5	-	-	135	60	175	359	-	9	-
50	7	-	1,361	-	-	24	-	-	-	-
50	8	-	440	-	-	17	-	-	-	-
51	1	-	12	-	8	201	-	-	-	10
51	2	34	542	59	-	9	-	-	-	-
51	3	5,096	37,392	49	3,936	152	-	-	-	-
51	11	-	-	1,322	1,606	5,287	2,270	215	81	-
51	12	130	512	-	-	741	3	-	2,917	-
51	14	480	9,083	954	6	159	-	-	-	12,305
51	15	1,180	1,740	-	-	110	176	-	-	-
51	16	48	-	-	-	-	-	-	-	-
51	17	65	25	-	14	9	37	6	-	-
51	18	10,862	16,943	480	1,870	878	321	62	37	362
51	20	87	12	-	-	1	5	7	-	-
51	22	5,902	29,464	430	466	67	102	5	28	-
51	25	1,007	393	72	2,127	1,492	-	-	4	-
51	27	4,125	7,270	90	670	166	229	-	-	-
51	32	352	4,766	1,025	-	-	-	-	-	-
51	33	-	-	-	-	21	-	-	-	-
51	34	21	296	-	-	26	-	-	-	-
51	36	-	-	-	-	74	-	-	-	-
51	37	-	-	-	-	-	-	-	16	-
51	38	231	8,210	626	-	351	-	13	290	-
52	1	-	-	-	-	-	6	5	-	-
52	2	167	-	-	-	2	-	-	-	-
52	3	634	2,141	120	-	80	201	-	14	45
52	4	370	250	-	-	-	-	-	4	-

USDA/APHIS

FY 1983

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
52	6	-	-	1,095	160	2,310	1,071	-	1,159	-
52	7	-	-	760	353	768	709	1,216	188	-
52	10	3,509	11,220	6,422	86	846	673	369	672	-
52	11	18,695	19,089	475	820	1,619	958	230	56	2
52	12	-	4,628	38	125	136	474	28	-	9
52	13	190	120	-	-	49	-	2	-	-
52	20	-	-	-	-	-	81	66	-	-
52	21	53	467	3,753	-	292	-	-	468	-
52	23	438	48	-	-	3	-	-	-	-
52	24	-	-	-	-	-	-	-	4	-
52	25	-	-	-	-	-	16	-	-	-
52	27	-	-	-	-	15	-	-	-	-
54	1	10,690	3,590	1,186	169	455	245	117	69	-
54	2	200	100	-	-	-	27	9	9	-
54	3	6	-	-	-	-	25	17	-	-
54	4	3,185	1,228	2	-	15	26	-	-	16
55	1	2,517	10,946	674	-	1,751	552	147	1,673	-
55	2	-	-	-	310	110	1	-	6	-
55	3	21,496	11,701	446	382	1,975	2,033	483	28	-
55	4	47,800	72,870	775	1,229	2,088	1,251	463	106	-
55	5	1,412	14,464	20	400	602	141	14	-	-
55	7	1,266	850	-	-	25	-	-	-	4
55	9	99,783	94,242	10,793	8,525	2,260	1,125	1,101	-	-
55	10	3,952	6,378	331	-	501	382	206	7	-
55	12	-	1,200	-	-	-	-	-	54	-
55	13	-	836	895	-	8	-	-	-	-
55	16	10	-	-	-	-	-	-	-	-
55	17	11,322	15,001	-	-	-	-	-	-	-
55	18	-	-	-	-	-	136	-	-	-
55	19	30	20	4	-	11	40	28	-	-
55	20	-	500	-	-	42	-	-	-	-
56	1	5,168	7,984	-	390	1,200	505	190	10	-
56	2	52	550	20	-	207	105	8	-	-
56	3	2,931	3,531	-	-	425	319	184	3	-
56	4	-	-	-	-	-	29	20	-	-
56	5	222	42	-	-	-	-	-	-	-
57	2	10,854	4,862	270	725	4,071	413	179	50	-
57	3	7,278	12,832	363	-	631	667	55	2,285	50
57	4	300	20	-	-	-	-	-	-	-
57	5	7,406	13,862	112	18,681	580	1,025	180	168	30
57	6	-	-	-	-	-	30	-	-	-
57	10	393	-	-	-	6	-	-	-	-
57	12	152	207	-	16	14	-	-	-	39
57	13	5,130	7,974	186	1,065	293	10	2	-	-
57	14	-	5,464	-	-	-	-	59	-	-
57	15	-	-	-	-	-	130	-	-	-
57	16	-	-	-	-	5	-	-	-	-

USDA/APHIS

FY 1983

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
58	1	5,597	3,135	13	284	64	49	135	29	-
58	2	2,595	-	38	-	1,086	112	-	-	-
58	3	22,509	26,232	577	363	1,407	1,249	409	99	-
58	5	60	500	26	101	33	107	-	-	-
58	7	-	-	285	133	1,712	346	280	342	-
58	9	296	-	-	-	22	68	-	-	-
58	15	3,485	9,234	48	795	46	886	298	9	12
58	16	35	40	-	-	25	-	-	-	-
58	17	6	30	-	30	19	1	-	-	-
58	19	-	1,658	1,000	-	288	-	-	-	-
58	20	335	2,500	-	-	5	-	-	-	11
58	21	-	-	-	-	-	-	-	62	-
58	22	40	-	-	-	-	3	-	-	-
58	23	-	18	-	-	1	18	18	-	-
58	24	-	-	-	-	12	-	-	-	30
58	26	-	-	-	-	-	-	-	-	1,945
58	28	-	1,210	-	-	571	-	-	-	-
58	29	-	-	-	-	-	-	-	-	4
58	32	-	280	281	-	-	-	-	-	-
58	33	1,500	500	-	-	-	-	-	-	-
58	34	-	725	12	-	-	-	-	-	-
58	35	-	-	-	-	-	-	12	-	-
58	36	-	430	-	-	-	-	-	-	-
58	37	-	-	24	-	-	-	-	-	-
58	43	200	-	-	-	22	-	12	-	-
58	46	-	-	-	-	-	70	4	-	-
58	48	-	-	-	-	10	-	-	-	-
58	49	-	-	-	-	2	55	3	-	-
58	50	-	-	-	-	4	29	-	-	-
58	52	-	-	-	-	-	10	-	-	-
58	53	64	-	-	-	-	-	-	-	-
58	999	-	3,285	-	-	27	-	-	-	-
61	1	5,171	13,613	669	168	394	583	101	5	-
61	2	17,232	19,627	470	1,878	766	534	332	12	-
61	4	2	-	-	-	3	9	10	-	-
61	6	-	1,600	-	-	-	-	-	-	-
61	7	-	-	-	-	31	-	-	-	-
61	15	-	-	-	-	-	44	-	-	-
63	1	4	60,605	3,741	10,295	1,580	330	164	-	4
63	2	925	7,537	379	-	86	64	3	4	-
63	3	1,077	2,446	-	-	160	15	-	95	-
63	4	1,629	29,106	322	275	819	2,617	97	8	32
63	5	28,133	10,696	347	989	2,205	1,498	37	223	-
63	6	48	520	10	-	23	-	13	18	-
63	7	2,909	20,165	8	-	117	-	-	-	-
63	8	268	10	6	-	16	-	-	-	-
63	10	570	1,333	440	-	819	-	-	-	-

USDA/APHIS

FY 1983

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
63	11	2,624	1,995	79	-	372	66	31	-	-
64	1	7,251	258,301	429	1,180	41	11	-	-	-
64	2	500	1,520	-	300	51	459	35	-	-
64	5	237	12	52	345	77	1,125	128	36	100
64	8	-	-	144	2,324	360	891	554	311	-
64	9	579	1,979	-	77	19	9	-	-	-
64	10	-	-	-	-	-	50	-	-	-
64	14	55	75	20	6	5	26	2	-	-
64	15	144	-	-	-	-	-	-	-	-
65	1	4,240	7,400	358	332	899	1,050	178	81	-
65	2	664	330	220	-	8	1	-	-	-
65	4	47	58	10	-	24	1	5	-	-
71	1	2,913	7,314	143	60	406	189	75	2	89
71	5	7,500	500	150	-	300	-	-	-	-
72	1	6,878	52	2	-	5	59	8	-	-
72	2	19,337	13,518	213	173	526	488	708	912	-
72	3	14,551	6,912	802	1,142	2,195	1,534	237	184	151
72	6	1,600	2,500	90	-	500	48	72	2,400	-
72	7	31	-	-	-	2	-	-	-	-
72	8	76	223	-	10	12	75	50	-	-
72	9	679	200	-	-	2	-	-	-	-
72	10	277	233	7	-	19	-	-	-	-
72	11	760	400	120	-	56	-	-	-	-
72	13	-	-	-	-	10	-	-	-	-
72	16	-	-	-	-	12	-	-	-	-
73	1	565	2,371	6	36	256	563	185	-	-
73	2	2,555	5,929	236	-	235	102	-	-	-
73	3	976	-	-	-	82	-	-	-	-
73	7	1,500	200	287	78	321	344	214	76	-
73	8	-	2,417	-	-	-	-	-	-	-
73	9	-	-	-	-	-	-	-	27	-
73	10	1,000	12	-	-	11	74	6	-	-
73	11	3,393	8,199	175	-	411	149	-	-	72
73	12	-	-	-	-	358	13	101	3	-
73	13	-	-	-	-	29	-	-	-	-
73	14	113	6	-	-	8	-	-	-	-
74	1	-	-	-	374	17	-	-	69	-
74	3	1,994	1,773	10	-	16	18	-	2,921	-
74	6	135	7,023	15	-	-	-	-	-	-
74	7	-	-	-	-	-	-	-	11	-
74	8	5	38	-	-	70	3	15	-	-
74	11	4,655	801	-	-	4,715	63	8	109	-
74	12	5,370	14,445	912	170	1,419	947	140	257	-
74	15	-	-	-	-	-	19	-	-	-
74	18	-	-	485	1,685	2,613	1,953	44	170	-
74	20	5,618	1,840	28	412	144	645	-	12	-
74	25	300	500	-	-	18	28	-	20	5

FY 1983

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
74	27	341	186	-	147	122	30	-	-	-
74	28	15	12	-	-	252	52	2	-	-
74	29	3,800	20,200	-	40	164	79	12	75	-
74	30	-	-	-	-	-	322	-	-	-
74	31	768	449	-	-	31	-	-	-	580
74	32	75	-	-	-	12	-	-	-	-
74	33	589	5,781	208	-	56	528	14	-	-
74	35	-	-	-	-	12	104	80	-	-
74	36	-	-	-	-	110	-	-	-	-
74	37	-	-	-	-	3	85	-	-	-
74	38	19	4,363	4	-	28	-	-	-	-
74	39	365	508	50	-	1	173	6	13	-
74	40	1,333	152	-	1,430	2	-	-	-	-
74	42	-	-	197	-	381	-	-	-	-
74	43	-	-	1,800	-	720	-	-	-	-
74	44	-	-	-	-	375	-	-	-	-
74	45	6	11	3	6	3	12	12	-	-
74	46	-	600	-	-	-	-	-	-	-
74	47	1,157	2,926	-	-	37	-	-	-	-
74	48	10	-	-	-	-	-	-	-	-
74	49	1,500	100	600	-	874	-	-	-	-
74	50	-	-	145	85	873	370	337	25	-
74	53	-	-	-	-	72	-	-	-	-
74	54	-	-	-	-	-	5	6	-	-
74	55	-	-	-	-	27	-	-	-	-
74	56	-	-	-	-	-	10	-	-	-
74	57	-	-	-	-	-	92	-	-	-
74	59	-	4,190	-	-	27	-	-	-	-
74	60	-	-	-	-	-	86	-	-	-
74	61	-	235	129	-	22	-	-	-	-
74	63	-	-	-	-	372	-	-	-	-
74	65	10,037	30,065	328	157	471	678	23	443	-
74	66	60	75	-	-	4	-	7	-	-
74	68	860	3,609	469	1,355	2,344	546	134	187	-
74	69	375	250	-	-	4	-	-	5	-
74	70	117	2,018	-	-	10	-	-	-	48
74	71	23,680	15,892	291	2,450	1,865	1,166	445	44	-
74	72	67,820	127,281	2,549	6,325	7,672	2,392	450	19	-
74	73	6,888	4,886	103	1,033	1,040	823	346	21	84
74	74	-	8	3	-	-	-	-	-	-
74	75	1,150	5,000	-	-	-	-	-	-	-
74	77	-	1,000	26	1,750	-	215	-	-	-
81	1	1,500	5,180	82	-	60	-	-	98	31
81	2	464	5,198	5	25	106	-	11	-	-
81	4	-	282	893	-	-	-	-	-	-
82	1	763	2,119	-	-	124	17	-	-	-
82	2	847	1,940	27	-	12	-	-	-	-

USDA/APHIS

FY 1983

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
82	4	-	10	-	-	3	-	-	-	-
83	1	-	-	42	706	38	-	-	-	-
83	2	-	-	-	-	3	-	-	-	-
83	4	-	-	2	-	2	30	8	-	-
84	1	1,289	43,029	705	1,308	1,508	-	21	-	-
84	3	4,206	4,651	659	20	389	1,732	289	-	958
84	5	-	-	-	-	-	17	10	-	-
84	6	22,556	42,997	454	278	3,283	682	303	178	-
84	7	-	-	1,129	1,050	731	24	121	-	-
84	8	32	-	-	-	16	-	-	-	-
84	10	-	908	942	-	20	-	-	-	-
84	11	10	-	-	-	8	-	-	-	-
84	12	1	-	2	1	8	4	6	-	-
84	13	4	3	3	-	2	24	27	-	3
84	14	238	76	-	-	-	-	-	-	-
84	16	887	100	-	-	-	418	-	-	-
84	21	23	6	-	-	8	-	-	-	-
84	24	-	8,550	-	-	432	-	-	-	-
84	27	-	-	-	-	-	9	48	-	-
84	29	-	-	-	-	84	-	-	-	-
84	31	-	-	-	-	8	-	-	-	-
84	32	-	5,000	-	-	-	-	-	-	-
84	34	-	-	-	-	41	55	-	-	-
84	35	-	150	-	-	7	-	-	-	-
84	36	-	35,654	2,618	18,636	1,269	570	112	-	-
85	1	1,093	2,357	36	125	52	-	-	24	-
85	2	4,912	13,816	260	-	278	351	91	156	69
85	3	8,372	2,527	182	1,211	3	1,221	-	66	-
85	7	600	100	-	-	-	-	-	-	-
85	9	944	11	20	49	34	-	-	648	-
85	11	415	241	-	-	-	-	-	301	-
85	12	60	1,085	-	-	4	67	-	-	-
86	2	2,491	10,248	30	537	320	165	13	-	11
86	3	22,161	15,700	774	1,247	2,618	506	298	128	-
86	5	110	40	4	-	7	-	10	-	-
86	6	600	-	2	150	-	17	14	32	-
86	9	-	-	25	-	116	151	-	-	-
86	10	6	-	-	-	-	43	-	-	-
86	11	-	-	-	-	-	27	-	-	-
86	13	483	247	199	-	284	-	-	-	-
86	14	-	747	-	-	41	-	-	-	-
86	15	-	-	-	-	-	24	-	-	-
87	1	10,721	84,332	1,776	146	2,137	672	318	-	-
87	2	2,364	30,440	379	205	201	60	4	-	-
87	3	556	1,253	58	48	38	26	13	2	-
87	4	72	110	-	-	-	-	-	-	-
87	5	-	-	250	-	150	-	-	-	-

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
87	6	-	-	-	-	-	13	-	-	-
87	8	15	50	-	-	12	1	-	-	-
87	9	552	11,760	135	-	2,902	-	-	-	-
87	10	710	-	450	3	570	75	-	-	-
87	11	-	-	-	-	-	8	-	-	-
88	1	1,178	6,385	576	34	105	107	18	-	-
91	1	22,380	27,742	429	2,872	2,413	993	342	1,872	12
91	2	-	-	68	2	634	1,452	389	40	-
91	3	-	-	-	-	-	199	-	-	-
91	5	150	3,250	-	-	-	-	11	-	-
91	6	20,499	9,437	341	-	15	462	15	-	-
91	7	197	660	192	-	95	49	25	5	-
91	9	582	1,089	13	13	17	-	-	2	20
91	11	95	9,830	10,667	-	70	-	-	-	-
91	13	-	6,603	-	-	-	-	-	-	-
91	15	-	-	-	-	-	-	-	35	-
91	17	-	-	-	-	-	-	2	10	500
91	18	-	-	-	-	-	17	18	-	-
91	19	-	-	-	-	-	20	-	-	-
91	20	80	650	-	-	19	-	-	-	-
91	23	960	-	-	-	96	-	-	5	-
91	25	1,796	25,286	316	830	259	145	-	-	-
92	1	7,013	8,983	578	50	411	540	103	33	-
92	2	35	-	-	-	103	-	164	6	-
92	3	-	-	-	-	-	-	19	-	-
92	5	7,414	6,058	14	27	480	164	16	7	-
92	6	400	500	-	-	2	42	-	-	-
92	7	4	24	-	-	12	-	-	-	-
92	8	2,400	6,000	6	38	46	-	99	47	-
92	10	412	403	10	171	68	-	-	2,405	-
92	12	540	-	-	-	-	-	-	-	-
92	13	-	-	-	-	31	-	-	-	-
92	15	-	-	-	-	-	12	18	-	-
92	16	-	-	23	-	-	-	-	-	-
92	18	297	-	-	40	4	-	-	-	53
92	20	-	-	-	-	256	-	-	-	-
92	22	-	-	-	-	-	5	3	-	-
93	3	-	-	-	-	-	1	17	-	-
93	4	20,766	69,071	797	817	6,451	1,108	705	872	-
93	6	1,722	5,967	47	45	155	89	101	25	-
93	7	230	3,500	150	300	60	48	6	-	-
93	9	786	2,191	18	-	18	65	-	-	-
93	11	-	-	16	-	258	1	-	-	-
93	13	30	-	-	-	2	-	-	-	-
93	15	1,720	81,000	800	31	1,900	-	-	63	-
93	16	4,999	1,923	8	298	1,792	221	45	5	-
93	17	103	4,357	6	-	222	44	3	9	-

USDA/APHIS

FY 1983

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
93	18	-	-	7,383	-	4,907	64	2	3	-
93	20	10,836	20,624	61	1,385	3,875	362	37	109	-
93	25	194	50,100	58	345	111	-	-	6	-
93	26	9,783	67,556	65	103	839	17	11	387	-
93	28	3,971	3,945	29	-	206	-	100	58	-
93	29	53,515	60,976	5,066	1,554	1,795	635	454	286	-
93	30	100	5,000	-	-	75	-	-	-	-
93	31	11,794	566	55	-	3,266	-	-	-	-
93	32	955	12	-	20	73	803	-	-	-
93	34	60	800	-	-	24	-	-	-	-
93	35	2	2,110	-	-	4	43	-	24	-
93	36	250	-	-	-	317	2	218	-	-
93	37	-	493	-	-	1	18	-	-	-
93	39	4,372	11,308	153	154	400	74	8	62	33
93	40	-	-	-	-	26	85	6	-	-
93	41	52	14,190	37	-	18	-	-	-	-
93	43	223	10	-	-	-	17	-	-	-
93	44	132,011	433,084	5,564	116,779	21,483	6,367	2,875	3,436	1,675
93	47	6	1,000	-	-	-	-	-	-	-
93	50	587	561	101	-	64	32	-	-	-
93	52	2,153	849	92	-	1,426	-	-	-	-
93	53	4	4	-	-	-	12	8	-	-
93	56	104	951	185	-	407	32	-	-	-
93	58	222	128	-	-	1	-	-	-	-
93	62	1,150	608	-	-	390	-	8	-	-
93	63	-	-	245	-	3,038	-	5	-	-
93	64	-	-	-	-	-	35	350	-	-
93	65	5,664	557	862	-	1,756	-	-	-	-
93	66	36,668	26,775	138	158	280	18	6	28	-
93	67	1,660	2,243	1,810	100	4,653	-	69	-	-
93	68	-	5,400	-	-	-	-	-	-	-
93	71	1,279	21,639	1,121	-	2,115	21	89	-	-
93	72	-	10,000	-	-	144	-	-	-	52
93	74	-	-	-	-	334	-	-	-	-
93	77	35	29	-	10	4	-	-	-	-
93	80	34	64	3	-	2	9	8	-	2
93	81	-	-	-	-	67	-	-	-	-
93	82	10	5	-	24	2	-	-	-	-
93	84	16	243	-	-	19	-	21	-	-
93	86	-	12,000	-	-	315	-	-	-	-
93	88	16	-	-	-	-	-	-	-	-
93	93	-	-	-	-	-	31	-	-	-
93	94	-	-	-	-	73	-	-	-	-
93	95	1,418	1,690	158	-	969	-	10	-	-
93	97	76	40	-	12	-	-	-	-	-
93	98	-	-	-	-	195	-	-	-	-
93	99	600	850	-	50	1	10	-	-	-

USDA/APHIS

FY 1983

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
93	100	-	-	-	-	36	-	-	-	-
93	103	250	40	-	-	-	-	-	-	-
93	104	176	410	574	-	-	-	-	-	-
93	105	262	26	-	132	56	-	-	-	-
93	106	-	291	13	-	-	-	-	-	-
93	117	81	150	16	-	3	-	1	-	-
93	118	-	-	-	-	133	-	154	-	-
93	121	-	7,740	149	-	1,412	17	-	-	-
93	122	21	975	150	-	-	-	-	-	-
93	123	-	-	-	-	107	-	-	-	-
93	124	100	200	1	1	15	150	318	-	-
93	126	46	79	-	-	-	-	-	-	-
93	127	-	-	-	-	36	-	-	-	-
93	130	2	10	-	1	-	-	-	-	-
93	131	42	445	-	-	3	-	-	-	-
93	133	-	611	-	-	-	-	-	-	-
93	134	-	-	-	-	-	-	-	13	-
93	135	-	-	-	-	7	-	-	-	-
93	136	-	-	-	-	-	4	5	-	-
93	138	112	15	-	-	-	-	-	-	-
93	140	-	-	-	-	45	-	-	-	-
93	141	-	-	-	-	58	-	-	-	-
93	144	-	-	-	-	7	-	-	-	-
93	145	12	29,670	439	-	3,196	-	-	-	-
93	147	-	575	-	-	921	-	-	-	-
93	149	12	20	8	-	4	30	20	-	-
93	151	2,350	150	11	17	7	-	1	23	-
93	153	-	-	-	-	-	-	-	26	-
93	155	-	3,027	1,981	-	7,289	-	-	-	-
93	156	-	-	-	-	-	56	20	-	-
93	157	2	-	-	-	10	-	-	-	-
93	158	-	119	-	-	465	-	-	-	-
93	159	1,103	-	-	-	546	-	-	-	-
93	160	9	-	-	-	-	-	-	-	-
93	161	1	-	-	-	17	-	-	-	-
93	165	82	1,136	-	-	31	-	-	-	-
93	166	-	-	-	-	-	15	-	-	-
93	169	1,460	-	-	-	745	-	-	-	-
93	171	-	-	-	-	-	22	-	-	-
93	173	-	-	-	-	131	-	-	-	-
93	174	-	-	-	-	104	-	-	-	-
93	176	-	61	-	-	53	-	-	-	-
93	180	72	332	-	-	22	-	-	-	-
93	181	3	700	550	-	2	-	-	-	-
93	183	-	-	520	-	-	-	-	-	-
93	184	-	-	-	-	136	-	-	-	-
93	186	-	-	-	-	224	-	15	-	-

USDA/APHIS

FY 1983

State	Site	Rats	Mice	Guinea Pigs	Hamsters	Rabbits	Dogs	Cats	Primates	Birds
93	187	-	-	-	-	-	-	-	13	-
93	188	-	-	-	-	25	-	-	-	-
93	189	800	852	25	-	418	-	-	-	-
93	191	-	-	-	-	-	5	14	-	-
93	193	-	-	-	-	-	25	41	-	-
93	196	-	-	-	-	40	-	-	-	-
93	197	464	72	-	-	25	-	-	-	-
93	198	1,250	100	150	-	2	-	-	-	-
93	199	9	-	15	5	314	-	-	-	-
93	203	600	800	-	-	25	-	-	-	100
94	1	-	-	-	225	119	395	31	2,059	-
94	2	-	-	-	-	31	-	-	-	-
94	4	-	-	-	-	-	-	-	43	-
94	5	-	-	-	-	134	-	-	-	-
94	8	-	-	-	-	5,500	-	-	-	-
94	10	-	-	-	-	-	-	10	-	-
95	2	3,085	2,770	416	2,400	275	21	293	51	-
95	3	2,434	158	-	-	88	3	4	50	-
96	1	6	40	20	-	-	1	-	-	-
FED	1	201,930	505,774	23,971	11,637	8,438	920	587	4,739	-
FED	2	55,276	143,779	28,869	5,534	3,919	839	218	680	172
FED	3	126,614	190,238	3,747	4,732	11,506	5,762	1,304	469	133
FED	4	45,011	120,941	2,854	2,396	2,936	2,015	626	1,348	466
FED	5	7,862	31,106	1,138	255	569	23	5	-	-
FED	6	3,036	79,501	5,869	11,331	2,411	36	34	33	3,568

(Appendix C addendum)

Registration Number Codes
for USDA/APHIS
reporting institutions

United States
Department of
Agriculture

Animal and
Plant Health
Inspection
Service

Veterinary
Services

March 1983

Animal Welfare: List of Registered Research Facilities FY 1983

ALABAMA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	546	AUBURN UNIVERSITY			AUBURN	36
A	5413	EAST ALABAMA EMERGENCY MEDICAL SERVICES, INC.		P.O. BOX 2331	ANNISTON	3620
A	5411	KEEVET LABORATORIES, INC.		P.O. BOX 1708	ANNISTON	36202
A	5412	NORTH ALABAMA EMERGENCY MEDICAL SERVICES, INC.		P.O. BOX 2104	DECATUR	35602
A	5414	SNEAD STATE JUNIOR COLLEGE		DRAWER D	BOAZ	35957
A	541	SOUTHERN RESEARCH INSTITUTE		2000 NINTH AVE. S.	BIRMINGHAM	35205
A	5410	THE JIM DANDY CO., INC.		P.O. BOX 10687	BIRMINGHAM	35202
A	549	THE UNIVERSITY OF ALABAMA		PO BOX 2846	UNIVERSITY	35486
A	542	TUSKEGEE INSTITUTE			TUSKEGEE	36088
A	544	UNIV. OF AL. IN BIRMINGHAM		1919 7TH AVE SOUTH	BIRMINGHAM	35233
A	548	UNIVERSITY OF SOUTH ALABAMA		307 GAILLARD BLVD.	MOBILE	36688

ALASKA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	9601	UNIVERSITY OF ALASKA			FAIRBANKS	99701

ARIZONA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	862	ARIZONA STATE UNIVERSITY			TEMPE	85281
A	8613	ARMOUR AND COMPANY	ARMOUR RESEARCH CENTER	15101 N SCOTTSDALE ROAD	SCOTTSDALE	85260
A	8611	CARDIOVASULAR RESEARCH PROGRAM		421 NORTH 18TH ST SUITE 115	PHOENIX	85006
A	8614	CENTRAL ARIZONA VETERINARY LAB. INC.		P.O. BOX 1298	CASA GRANDE	85222
A	865	NORTHERN ARIZONA UNIVERSITY			FLAGSTAFF	86001
A	866	ST. JOSEPH'S HOSP & MED. CTR		350 W. THOMAS RD.	PHOENIX	85113
A	8610	ST. LUKE'S HOSPITAL MED CTR		1820 E. POLK	PHOENIX	85006
A	863	UNIVERSITY OF ARIZONA			TUCSON	85721
A	868	W. L. GORE & ASSOCIATES, INC.		1505 N. FOURTH STR. BOX 1300	FLAGSTAFF	86002

ARKANSAS

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	713	ANIMAL BEHAVIOR ENTERPRISES, INC.		4600 ALBERT PIKE	HOT SPRINGS	71901
A	711	UNIVERSITY OF ARKANSAS			FAYETTEVILLE	72

CALIFORNIA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	93180	ADVANCED GENETICS RESEARCH INST.		2220 LIVINGSTON ST. SUITE 202	OAKLAND	94606
A	9367	ALLERGAN PHARMACEUTICALS		2525 DUPONT DR.	IRVINE	92664
A	9367	ALPHA GAMMA LABS		180 E. MONTACITO AVE.	SIERRA MADRE	91024
A	93155	ALPHA THERAPEUTIC CORP		5555 VALLEY BLVD	LOS ANGELES	90032
A	9366	ALZA CORPORATION		950 PAGE MILL RD.	PALO ALTO	94304
A	93121	AMERICAN EDWARDS LABORATORIES		17221 RED HILL AVE.	SANTA ANA	92705
A	9371	AMERICAN MCGAW		2525 MCGAW AVE	SANTA ANA	92711
A	9363	AMERICAN PHARMASEAL		4401 FOXDALE AVE.	IRWINDALE	91706
A	93176	AMERICAN QUALEX INCORPORATED		14620 FIRESTONE BLVD.	LA MIRADA	90638
A	93158	ANTIBODIES INCORPORATED		BOX 442	DAVIS	95616
A	9362	APPLIED BIO. SCI. LAB., INC		5320 SAN FERNANDO RD	GLENDALE	91201
A	93175	ARMOS CORPORATION		180 KIMBALL WAY	SOUTH SAN FRANCISCO	94080
A	93181	AXONICS, INC.		1500 SALADO DR. SUITE 202	MOUNTAIN VIEW	94043
A	9382	BARNES-HIND PHARMACY, INC.		985 KIFER RD.	SUNNYVALE	94086
A	9374	BECKMAN INSTRUMENTS, INC.		2041 E. LAMBERT RD.	LA HABRA	90631
A	9383	BENTLEY LABORATORIES, INC.		17502 ARMSTRONG AVE.	IRVINE	92705
A	93141	BIO-RAD LABORATORIES		2200 WRIGHT AVE.	RICHMOND	94804
A	9372	BIO-SCIENCE LABORATORIES		7600 TYRONE AVE.	VAN NUYS	91405
A	9386	BIO-TECHNICS LABS., INC.		1133 CRENSHAW BLVD.	LOS ANGELES	90019
A	93106	BUREAU OF PUBLIC HEALTH LAB.		313 N. FIGUEROA ST.	LOS ANGELES	90012
A	9389	C.R. DREW POST-GRAD MED. SCH		1621 E. 120TH ST.	LOS ANGELES	90059
A	93125	CALBIOCHEM-BEHRING CORP	SARGEANT FARMS	10933 NORTH TORREY PINES	LA JOLLA	92037
A	9325	CALIFORNIA INSTITUTE OF TECH.		1201 E CALIF. BLVD.	PASADENA	91125
A	9338	CALIFORNIA STATE COLLEGES		5670 WILSHIRE BLVD.	LOS ANGELES	90036
A	9332	CEDARS-SINAI MEDICAL CENTER		8720 BEVERLY BLVD.	LOS ANGELES	90048
A	9330	CHILDREN'S HOSP MEDICAL CTR.		51ST & GROVE STREETS	OAKLAND	94609
A	9334	CHILDREN'S HOSPITAL OF SAN FRANCISCO		P.O. BOX 3805	SAN FRANCISCO	94119
X	9311	CHILDRENS HOSPITAL OF LOS ANGELES		4650 SUNSET BLVD.	LOS ANGELES	90027
A	93184	CIRCLE EWE RANCH/PLOK, VELMA		30250 SAN TIM CYRNO	REDLANDS	92373
A	937	CITY OF HOPE MEDICAL CENTER		1500 E. DUARTE RD.	DUARTE	91010
A	93128	CLAREMONT MEN'S COLLEGE		11TH & DARTMOUTH AVENUES	CLAREMONT	91711
A	93144	CLINICAL IMMUNO-DIAGNOSTICS, INC.		1378-D LOGAN AVE.	COSTA MESA	92626
X	93111	CLMG, INC.		2222 OCEAN VIEW AVENUE	LOS ANGELES	90057
A	93157	COLLEGE OF OSTEOPATHIC MEDICINE OF THE PACIFIC		309 POMONA MALL	POMONA	91766
A	93118	COMPTON COMMUNITY COLLEGE		1111 E. ARTESIA BLVD	COMPTON	90221
A	9380	CONSUMNES RIVER COLLEGE		8401 CENTER PARKWAY	SACRAMENTO	95823
A	93147	COOPERVISION INC.		455 EAST MIDDLEFIELD RD	MOUNTAIN VIEW	94
A	93133	COUNTY OF SAN DIEGO		5555 OVERLAND AVE BLDG. 4	SAN DIEGO	92
A	93148	CRYOVITA LABORATORIES, INC.		UNIT #304 4030 N. PALM ST.	FULLERTON	92
A	9316	CUTTER LABORATORIES, INC.		4TH & PARKER STS.	BERKELEY	9471
A	93182	DAVIDSON, JEFFREY N.	HEALTH MAAGEMENT SERVICES	P.O. BOX 2046	TULARE	9327
A	93104	DIAGNOSTIC DATE, INC.		518 LOGUE AVE	MOUNTAIN VIEW	940

CALIFORNIA—Continued

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	93161	E-Y LABS, INC		127 N AMPHLETT	SAN MATEO	94401
A	93186	EL-LABS		4003 CHERRYVALE AVE.	SOQUEL	95073
A	9364	ENDOCRINE SCIENCES		18418 OXNARD ST.	TARZANA	91356
X	93101	ESTELLE DOHENY EYE FOUNDATION		1355 SAN PABLO ST.	LOS ANGELES	90033
A	93136	FOOTHILL COLLEGE		12345 EL MONTE RD.	LOS ALTOS HILLS	94022
A	93153	GIBBONS & GALLINACEOUS BD CTR		BOX 93	SAUGUS	91350
A	93138	GROSSMONT COMMUNITY COLLEGE		5800 GROSSMONT COLLEGE DR.	EL CAJON	92020
X	9366	HOAG MEMORIAL HOSPITAL PRESBYTERIAN		301 NEWPORT BLVD	NEWPORT BEACH	92660
A	93163	HUBBS/SEA WORLD RESEARCH INST		1700 SOUTH SHORES RD	SAN DIEGO	92109
A	93168	HUMAN/DOLPHIN FOUNDATION	MARINE WORLD	BOX 4172	MALIBU	90265
A	9336	HUNTINGTON INSTITUTE OF APPLIED MEDICAL RES.		734 FAIRMOUNT AVE.	PASADENA	91105
A	93164	HUTTON, D	CLONAL RESEARCH LTD	1802 MONROVIA AVE	NEWPORT BEACH	92663
A	9335	INST MED/RES SANTA CLARA CO		751 S. BASCOM AVE.	SAN JOSE	95128
A	9317	INSTITUTE OF MEDICAL SCIENCES		2200 WEBSTER ST., RM 310	SAN FRANCISCO	94115
A	93113	INTER SCIENCE INST.		2000 COTNER AVE.	LOS ANGELES	90025
A	9388	INTERNATIONAL MED SYSTEMS LTD		1886 SANTA ANITA AVE.	SOUTH EL MONTE	91733
A	93132	JANUS LAB.		P.O. BOX 2026	EL CAJON	92021
A	93135	KAISER FOUNDATION HOSPITALS		ORDWAY BLDG.	OAKLAND	94666
A	93109	KEARLEY, D.V.M., EDWARD G.		690 QUINCY RD.	TURLOCK	95380
A	93122	L. PAULING INST. OF SCI & MED		440 PAGE MILL RD.	PALO ALTO	94306
A	93165	LA JOLLA CANCER RESEARCH FOUND		2945 SCIENCE PARK RD	LA JOLLA	92037
A	9388	LA VERNE COLLEGE		1950 THIRD ST.	LA VERNE	91750
A	9346	LEE PHARMACEUTICALS		1444 SANTA ANITA AV.	SOUTH EL MONTE	91733
A	93152	LITTLE, JF	FELINE HOME STUDIES	4640 OLNEY ST	SAN DIEGO	92109
A	936	LOMA LINDA UNIVERSITY			LOMA LINDA	92354
A	9353	LOS ANGELES PIERCE COLLEGE		5201 WINNETKA BLVD.	WOODLAND HILLS	96413
A	9362	LOYOLA UNIVERSITY		7101 W 80TH ST.	LOS ANGELES	90045
A	93174	MAURRY BIOLOGICAL CO., INC.		8108 S. WESTERN AVE.	LOS ANGELES	90047
A	9368	MEDI-PHYSICS, INC.		5866 CHRISTIE AVE.	EMERYSVILLE	94608
A	9343	MEMORIAL HOSP. OF LONG BEACH		2801 ATLANTIC AVE.	LONG BEACH	90806
X	9390	MERCY SAN JUAN HOSPITAL		6501 COYLE AVE.	CARMICHAEL	95608
A	939	MOUNT ZION HOSPITAL AND MEDICAL CENTER		1600 DIVISADERO ST.	SAN FRANCISCO	94115
A	93117	MT. SAN ANTONIO COLLEGE		1100 N. GRAND AVE.	WALNUT	91789
A	93110	MURPHY, HOWARD F		400 N. LA COSTA DR.	CARLSBAD	92008
A	9369	NEWPORT PHARM. INTL., INC.		1590 MONROVIA BLVD.	NEWPORT BEACH	92660
X	9392	NICHOLS INST./ENDOCRINOLOGY		1300 S. BEACON ST	SAN PEDRO	90731
A	93145	NORTH AMERICAN SCIENCE ASSOC. OF CA., INC.		1582 DEERE AVE	IRVINE	92714
A	93105	OCCIDENTAL COLLEGE		1600 CAMPUS RD.	LOS ANGELES	90041
A	9345	OLIVE VIEW MEDICAL CENTER		14445 OLIVE VIEW DRIVE	SYLMAR	91342
A	93149	ORANGE COAST COLL. ANIMA HLTH. TECHNOLOGOY PROG.		2701 FAIRVIEW RD.	COSTA MESA	92626
A	93158	ORTHOPAEDIC HOSPITAL		2400 SO FLOWER ST	LOS ANGELES	90007
A	93166	OXIMETRIC INC		1212 TERRA BELLA AVE	MOUNTAIN VIEW	94043
A	9341	PALO ALTO MED. RES. FOUND.		860 BRYANT ST.	PALO ALTO	94301
A	93173	PANTEX DIVISION OF BIO-ANALYSIS		1737 21ST STREET	SANTA MONICA	90404
A	93103	PASADENA CITY COLLEGE		1570 E. COLORADO BLVD.	PASADENA	91106
A	9324	PASADENA FOUND./MEDICAL RES.		99 N. EL MOLINO AVE.	PASADENA	91101
A	93160	POINT LOMA COLLEGE	ANNEX LABORATORY	3900 LOMALAND DRIVE	SAN DIEGO	92106
A	9384	POMONA COLLEGE			CLAREMONT	91711
A	9369	RADICASSAY SYSTEMS LAB., INC.		1511 E. DEL AMO BLVD.	CARSON	90746
A	9337	RANCHO LOS AMIGOS HOSP. INC.		7413 GOLONDRINAS ST.	DOWNEY	90242
A	93107	RANCHO SANTIAGO COMM COL DIST		17TH ST AT BRISTOL	SANTA ANA	92706
X	9381	REDKEN LABORATORIES, INC.		14721 CALIFA ST.	VAN NUYS	91401
A	9316	RES. & EDU. INST. INC./HARBOR-UCLA MEDICAL CNTR.		1124 W. CARSON ST.	TORRANCE	90502
A	9369	SALK INST. FOR BIO. STUDIES		PO BOX 1809	SAN DIEGO	92112
A	93108	SAN BERNARDINO VALLEY COLLEGE		701 SO. MT. VERNON AVE.	SAN BERNARDINO	92403
X	9363	SAN DIEGO BIO-MED. RES. INST.		2802-1ST AVE. RM 104	SAN DIEGO	92103
A	93124	SAN DIEGO MESA COLLEGE		7250 MESA COLLEGE DR.	SAN DIEGO	92111
A	93143	SAN DIEGUITO ANIMAL CARE CTR.		PO BOX 84	RANCHO SANTE FE	92067
A	9347	SANSUM CLINIC RES. FOUND.		2219 BATH ST.	SANTA BARBARA	93102
X	93114	SANTA BARBARA CITY COLLEGE		721 CLIFF DR.	SANTA BARBARA	93109
A	93169	SCIENCE APPLICATIONS, INC.		484 PROSPECT ST PO. BOX 1464	LAJOLLA	92038
A	9315	SCRIPPS CLINIC & RES. FOUND.		476 PROSPECT ST.	LA JOLLA	92037
A	9313	SHELL DEVELOPMENT COMPANY		P.O. BOX 4248	MODESTO	95353
A	93140	SMITH KLINE INSTRUMENTS, INC.		486 POTRERO AVE.	SUNNYVALE	94086
A	93150	SOCIETY OF SCIENTIFIC CARE, INC.		PO BOX 7535	SAN DIEGO	92107
A	931	SONOMA STATE HOSPITAL			ELDRIDGE	95431
A	93185	SOO WOONG LEE D.D.S.		11618 BAIRD AVE.	NORTHRIDGE	91328
A	93127	SOUTHERN CALIFORNIA COLLEGE OF OPTOMETRY		2001 ASSOCIATED RD	FULLERTON	92631
A	93130	SOUTHWESTERN COLLEGE		900 OTAY LAKES RD.	CHULA VISTA	92010
A	93118	ST. JOHN'S HOSP. & HEALTH CTR.		1328 22ND ST.	SANTA MONICA	90404
A	9342	ST. MARY'S HOSP. MEDICAL CTR.		2200 HAYES ST.	SAN FRANCISCO	94117
A	9365	STANDARD OIL COMP. OF CA.		578 STANDARD AVE., RM 5201	RICHMOND	94802
A	9339	STANFORD RESEARCH INSTITUTE		333 RAVENSWOOD AVE.	MENLO PARK	94025
A	934	STANFORD UNIVERSITY		STANFORD MEDICAL CTR. L0030	STANFORD	94305
A	9364	STAR-KIST FOODS, INC.		582 TUNA ST.	TERMINAL ISLANDS	90731
A	9325	STATE OF CA/DEPT. OF HEALTH		2151 BERKELEY WAY	BERKELEY	94704
A	9331	STAUFFER CHEMICAL COMPANY		1200 SOUTH 47TH ST	RICHMOND	94804
A	93172	SULLIVAN, ARTHUR		5525 N. SULTANA AVE., APT 8	TEMPLE CITY	91780
A	93134	SUMMERS, JOHN E.		4100 HOLLISTER AVE.	CARMICHAEL	95608
A	933	SUTTER COM HOSP OF SACRAMENTO		52ND & F STS.	SACRAMENTO	95816
A	93120	SYLVAN CORNERS VET. HOSPITAL		7548 OLD AUBURN BLVD	CITRUS HEIGHTS	95610
A	9329	SYNTEX (U.S.A.) INC.		3401 HILLVIEW AVE.	PALO ALTO	94304
A	9361	SYVA CORPORATION		3151 PASTEUR DR.	PALO ALTO	94304
A	9373	TERA PHARMACEUTICALS, INC.		6820 STANTON AVE.	BUENA PARK	90621
A	93162	THE GORILLA FOUNDATION		PO BOX 3002	STANFORD	94305
X	935	THE HINE LABORATORIES, INC.		TEHAMA ST	SAN FRANCISCO	94103
A	9344	THE REGENTS OF THE UNIV OF CA		UNIVERSITY HALL	BERKELEY	94720
A	93171	THORATEC LABORATORIES CORP		2023 EIGHTH STREET	BERKELEY	94710
A	93154	UNILAB RESEARCH	E S UNILAB RESEARCH, INC.	2900 SEVENTH ST	BERKELEY	94710
A	93131	UNIVERSITY OF SAN DIEGO		ALCALA PARK	SAN DIEGO	92110
A	9377	UNIVERSITY OF SAN FRANCISCO			SAN FRANCISCO	94117
A	9320	UNIVERSITY OF SOUTHERN CALIFORNIA		2011 ZONAL AVE.	LOS ANGELES	90033
A	9390	UNIVERSITY OF THE PACIFIC		751 BROOKSIDE RD	STOCKTON	95207
A	9364	VALLEY CHLDNS HOSP & GUID CTR		3151 N. MILLBROOK	FRESNO	93703

CALIFORNIA—Continued

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	P CC
X	93129	VASCOR		4633 E. LA PALMA AVE.	ANAHEIM	9
A	93115	VICTOR VALLEY COLLEGE			VICTORVILLE	9
A	93100	VITAMINERALS, INC.		1815 FLOWER ST.	GLENDALE	9
A	93156	WESTERN COLLEGE OF ALLIED HEALTH CAREERS		4000 EL CAMINO AVE	SACRAMENTO	9
A	9340	WHITE MEMORIAL MEDICAL CENTER		1720 BROOKLYN AVE	LOS ANGELES	9
A	9387	WHITTIER COLLEGE			WHITTIER	9
A	93179	WILD ANIMAL TRAINING CENTER		P.O. BOX 1052	RIVERSIDE	9
A	9379	YUBA COLLEGE		LINDA & BEALE ROADS	MARYSVILLE	9
A	93151	ZOOLOGICAL SOCEITY OF SAN DIEGO		PO BOX 551	SAN DIEGO	9

COLORADO

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CC
A	8432	AMC CANCER RESEARCH CENTER AND HOSPITAL		6401 WEST COLFAX AVE	LAKEWOOD	8
A	8419	ARAPAHOE COMMUNITY COLLEGE		5900 S. SANTA FE DR	LITTLETON	8
A	8413	BEL RAE INSTITUTE		9870 E. ALAMEDA AVE.	DENVER	8
A	8427	CHILDREN'S HOSPITAL		1056 EAST 19TH AVE.	DENVER	8
A	8424	COBE LAB/ANIMAL LABORATORIES		1201 OAK ST.	LAKEWOOD	8
A	8412	COLORADO MOUNTAIN COLLEGE			GLENWOOD SPRINGS	8
A	847	COLORADO SERUM COMP		4950 YORK ST.	DENVER	8
A	8416	DENVER DEPT OF HEALTH & HOSP		WEST 8TH AVE. & CHEROKEE ST.	DENVER	8
A	8428	ELARS BIO RESEARCH LAB.		225 COMMERCE DR.	FORT COLLINS	8
A	8421	FORT LEWIS COLLEGE			DURANGO	8
A	8410	MERIDIAN BIO-MEDICAL, INC.		3278 S. WADSWORTH	DENVER	8
A	841	NAT'L JEWISH HOSP		3800 EAST COLFAX AVE	DENVER	8
A	8423	OTERO JUNIOR COLLEGE		18TH & COLORADO AVE.	LA JUNTA	8
A	8415	PENROSE HOSPITAL		2215 N. CASCADE AVE.	COLORADO SPRINGS	8
A	8420	REGIS COLLEGE		WEST 50TH & LOWELL BLVD.	DENVER	8
A	846	ST. JOSEPH HOSPITAL		1835 FRANKLIN ST.	DENVER	8
A	8414	THE COLORADO COLLEGE			COLORADO SPRINGS	8
A	848	UNIV OF NORTHERN COLORADO			GREELEY	8
A	848	UNIVERSITY OF COLORADO			BOULDER	8
A	8431	VALLEYLAB INC		5920 LONGBOW DR	BOULDER	8
A	8433	VIPONT CHEMICAL CO.		220 EAST OLIVE	FORT COLLINS	8
A	8429	WESTERN RESEARCH CORP.		2300 N. HWY 287, PO BOX 1255	FORT COLLINS	8
A	8411	WESTERN STATE COLLEGE OF COLORADO			GUNNISON	8
A	8434	WILDLIFE VACCINES, INC.		11475 WEST 48TH AVE.	WHEAT RIDGE	8

CONNECTICUT

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	CC
A	1629	BOEHRINGER INGELHEIM LTD.		90 EAST RIDGE, P.O. BOX 368	RIDGEFIELD	0
A	1630	CANINE RESEARCH FACILITY		PARKER BRIDGE ROAD	COVENTRY	0
A	1616	CT. STATE DEPT. OF HEALTH		PO BOX 1689	HARTFORD	0
A	1632	ENVIRONMENTAL HEALTH CNTR/ STAUFFER CHEMICAL CO.		400 FARMINGTON AVE.	FARMINGTON	0
A	1623	FAIRFIELD UNIVERSITY			FAIRFIELD	0
A	167	HARTFORD HOSPITAL		80 SEYMOUR ST.	HARTFORD	0
A	164	JOHN B. PIERCE FOUNDATION LABORATORY		290 CONGRESS	NEW HAVEN	0
A	1624	NEW ENGLAND INST., INC.		90 GROVE ST.	RIDGEFIELD	0
A	1626	QUINNIPIAC COLLEGE		555 NEW ROAD	HAMDEN	0
A	1631	SEA RESEARCH FOUNDATION, INC.	MYSTIC MARINELIFE AQUARIUM		MYSTIC	0
A	162	ST. FRANCIS HOSP. RES. LAB.		114 WOODLAND ST.	HARTFORD	0
A	1619	ST. MARY'S HOSPITAL		56 FRANKLIN ST.	WATERBURY	0
A	168	THE UNIVERSITY OF CONNECTICUT			STORRS	0
A	1628	UNITED STATES SURGICAL CORP.		150 GLOVER AVE.	NORWALK	0
A	1625	UNIV OF CT HEALTH CENTER		CENTER FOR LAB ANIMAL CARE	FARMINGTON	0
A	1611	UNIVERSITY OF HARTFORD		200 BLOOMFIELD AVE.	W. HARTFORD	0
A	161	YALE UNIVERSITY		333 CEDAR ST.	NEW HAVEN	0

DELAWARE

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CC
A	508	ALFRED I. DU PONT INSTITUTE		ROCKLAND RD.	WILMINGTON	
A	50R7	DU PONT CLINICAL SYSTEMS		BLDG. 300 ROUTE 896	GLASGOW	
A	508	DU PONT EXPERIMENTAL STATION		NEW MURPHY RD.	WILMINGTON	
A	501	E.I. DUPONT DE NEMOURS & CO.		PO BOX 30	NEWARK	
A	503	ICI AMERICAS, INC.		CONCORD PIKE & NEW MURPHY RD.	WILMINGTON	
X	505	STERWIN LABORATORIES, INC.		DUPONT HWY.	MILLSBORO	
A	504	UNIVERSITY OF DELAWARE			NEWARK	
X	506	WILMINGTON MEDICAL CTR., INC.		14TH & WASHINGTON STS.	WILMINGTON	

DISTRICT OF COLUMBIA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CC
A	DC7	AMERICAN UNIVERSITY		MASSACHUSETTS & NEBRASKA AVES.	WASHINGTON	2
A	DC3	CHILDREN'S HOSP. OF THE D.C.		2125-13TH ST., NW.	WASHINGTON	2
A	DC5	GEORGE WASHINGTON UNIVERSITY			WASHINGTON	2
A	DC4	GEORGETOWN UNIVERSITY		3900 RESERVOIR RD. N.W.	WASHINGTON	2
A	DC9	HOWARD UNIVERSITY/COLLEGE OF MEDICINE		2400 6TH ST. NW.	WASHINGTON	2
A	DC2	JACKSON LABORATORIES, INC.		2812-28TH ST NE PO BOX 10238	WASHINGTON	2
A	DC8	NATIONAL FOOD PROCESSORS ASSOCIATION		1133-20TH ST., N.W.	WASHINGTON	2
A	DC1	WASHINGTON HOSPITAL CENTER		GEORGE HYMAN MEMORIAL RESEARCH	WASHINGTON	2

FLORIDA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	5843	ALL CHILDREN'S HOSPITAL		801 6TH STREET SOUTH	ST PETERSBURG	33701
A	5832	BARRY LABORATORIES, INC.		461 N.E. 27TH ST.	POMPANO BEACH	33064
A	584	BIGGERT'S SMALL ANIMAL HOSP		2833 S. 4 ST.	FORT PIERCE	33450
A	5824	CHEMIE HAUS, INC.		180 CONCORD DR.	CASSELBERRY	32707
A	5844	CLEARWATER MARINE SCIENCE CENTER		249 WINDWARD PASSAGE	CLEARWATER	33615
A	5819	CORDIS CORP		PO BOX 370428	MIAMI	33137
A	5846	CRITIKON, INC.		1410 NORTH WESTSHORE BOULEVARD	TAMPA	33607
A	5828	DADE DIV AM HOSP SUPPLY CORP		PO BOX 520672	MIAMI	33152
A	582	DAWSON RESEARCH CORP		PO BOX 8272	ORLANDO	32809
A	5810	ECKERD COLLEGE		PO BOX 12560	ST PETERSBURG	33733
A	5826	FL INJURED WILDLIFE SANC. INC		BOX 260,RT 1	MELBOURNE	32935
A	5812	FLORIDA A&M UNIVERSITY			TALLAHASSEE	32307
A	5835	FLORIDA ATLANTIC UNIVERSITY			BOCA RATON	33431
A	5820	FLORIDA INST. OF TECHNOLOGY		BOX 1150 COUNTRY CLUB ROAD	MELBOURNE	32901
A	5836	FLORIDA INTERNATIONAL UNIV		TAMIAMI TRAIL	MIAMI	33199
A	5817	FLORIDA MED. ENTOMOLOGY LAB.		PO BOX 520-OSLO RD.	VERO BEACH	32960
A	581	FLORIDA STATE UNIVERSITY			TALLAHASSEE	32306
A	5845	INSTITUTE FOR DELPHINID RES.		PO. BOX DOLPHIN	MARATHON SHORES	33052
A	5831	JACKSON VETERINARY PRACTICE		RFD 5 BOX 9	ST. AUGUSTINE	32084
A	5838	KNOWLES ANIMAL CLINIC		1000 NW 27TH AVE	MIAMI	33125
A	5837	LEO GOODWIN INST FOR CANC RES		3301 COLLEGE	FT LAUDERDALE	33314
A	5847	LIFE SCIENCES, INC.		1508 49TH STREET SOUTH	ST PETERSBURG	33707
				3301 COLLEGE	FT LAUDERDALE	33314
A	5847	LIFE SCIENCES, INC.		1508 49TH STREET SOUTH	ST PETERSBURG	33707
A	5821	MANNHEIMER PRIMATE FOUND.		20256 S.W. 380TH ST.	HOMESTEAD	33030
A	5848	MEDICAL RESEARCH FOUNDATION OF ST. PETERSBURG, INC		501 11TH ST. N	ST PETERSBURG	33706
A	589	MIAMI HEART INSTITUTE		4701 N. MERIDIAN AVE.	MIAMI BEACH	33140
A	5841	MOTE MARINE LABORATORY, INC.		1600 CITY ISLAND PARK	SARASOTA	33577
A	586	MT. SINAI HOSPITAL RES. LAB.		4300 ALTON RD.	MIAMI BEACH	33140
A	5829	N. AMERICAN BIOLOGICALS, INC.		16500 NW 15 AVE.	MIAMI	33169
A	5840	NATIONAL BIOLOGICALS, INC		13710 48TH STREET NORTH	CLEARWATER	33520
A	5833	PAPANICOLAOU CANCER RES INST		1155 NW 14TH ST PO BOX 236188	MIAMI	33123
A	5822	ROLLINS COLLEGE			WINTER PARK	32789
A	5834	SANDERS MED. RES. FOUND. INC.		33 SE 3RD ST.	BOCA RATON	33432
A	5825	SERALC CORPORATION		15980 N.W. 15 AVE.	MIAMI	33169
A	5842	SHANK, J		3225 NO ANDREWS AVE	FT LAUDERDALE	33309
A	5814	SHERWOOD MEDICAL INDUSTRIES INC.		PO BOX 2078	DELAND	32720
A	5850	SOUTHEASTERN COLLEGE OF OSTEOPATHIC MEDICINE		1750 N.E. 168TH STREET	NORTH MIAMI BEACH	33162
A	5823	ST. PETERSBURG JR. COLLEGE		PO BOX 13489	ST. PETERSBURG	33733
A	5830	STATE OF FL., DIV. OF HEALTH		4000 W. BUFFALO AVE	TAMPA	33614
A	5816	UNIV. OF CENTRAL FLORIDA		PO BOX 25000,ALAFAYA TRAIL	ORLANDO	32816
A	5849	UNIVERSITY HOSPITAL BUILDING, INC.	MEMORIAL HOSPITAL OF JACKSONVILLE	3625 UNIVERSITY BLVD. SOUTH	JACKSONVILLE	32216
A	583	UNIVERSITY OF FLORIDA		OFFICE OF THE PRESIDENT	GAINESVILLE	32601
A	587	UNIVERSITY OF MIAMI			CORAL GABLES	33124
A	5815	UNIVERSITY OF SOUTH FLORIDA		4202 FOWLER AVE.	TAMPA	33620

GEORGIA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	5713	ALTANTA UNIV. CENTER		360 WESTVIEW DR. SW	ALTANTA	30314
A	573	EMORY UNIVERSITY			ATLANTA	30322
A	579	GEORGIA COLLEGE			MILLEDGEVILLE	31061
A	5710	GEORGIA INST. OF TECHNOLOGY			ATLANTA	30332
A	5712	GEORGIA STATE UNIVERSITY		UNIVERSITY PLAZA	ATLANTA	30303
A	574	MERCER UNIVERSITY SYSTEM			MACON	31207
A	5714	MERIEUX INSTITUTE		200 CEDAR CREEK DR.	ATHENS	30605
A	5711	OXFORD CHEMICALS	DIVISION OF CONSOLIDATED FOODS.	PO BOX 80202	ATLANTA	30366
A	578	PALMER CHEM. & EQUIP. CO. INC		PO BOX 867	DOUGLASVILLE	30134
A	575	UNIVERSITY OF GEORGIA SYSTEM			ATHENS	30601

HAWAII

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	9612	HAWAII DEPT OF AGRICULTURE		1428 S. KING ST.	HONOLULU	96814
A	963	QUEEN'S MEDICAL CENTER		PO BOX 861	HONOLULU	96808
A	9611	RES. CORP OF THE UNIV OF HI.		1110 UNIVERSITY AVE.	HONOLULU	96817
A	957	ST. FRANCIS HOSP RES LAB		2260 LILIKA ST.	HONOLULU	96817
A	962	UNIVERSITY OF HAWAII/LABORATORY ANIMAL SERVICE		2536 THE MALL, SNYDER HALL	HONOLULU	96822

IDAHO

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	8201	IDAHO STATE UNIVERSITY			POCATELLO	83201
A	8203	LONG, CO		101 N. GARDEN ST.	BOISE	83704
A	8204	NORTHWEST NAZARENE COLLEGE			NAMPA	83651
A	8202	UNIVERSITY OF IDAHO			MOSCOW	83843

ILLINOIS

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP
A	3358	A.E. STALEY MFG. CO.		22ND & ELDORADO BLDG # 60	DECATUR	62525
A	3325	ABBOTT LABORATORIES		1400 SHERIDAN RD.	NORTH CHICAGO	60064
A	3369	AMERICAN DENTAL ASSOCIATION		211 EAST CHICAGO AVE.	CHICAGO	60611
A	3320	ARGONNE NATIONAL LABORATORY		9700 SOUTH CASS AVE.	ARGONNE	60439
A	3333	ARMOUR PHARMACEUTICAL CO.		US ROUTE 54	BRADLEY	60915
A	3334	ARNAR-STONE LAB.		1500 WAUKEGAN RD.	MCGAW PARK	60085
X	3379	BIOLABS, INC.		2910 MACARTHUR BLVD.	NORTHBROOK	60062
A	3352	BLACKBURN COLLEGE			CARLINVILLE	62626
A	3386	BORDEN INC.		RR 1	ELGIN	60120
A	3359	BRADLEY UNIVERSITY			PEORIA	61606
A	3372	CHICAGO BOARD OF HEALTH		CHICAGO CIVIC CENTER	CHICAGO	60602
A	3310	CHICAGO COLLEGE OF OSTEOPATHY		1122 E. 53 ST.	CHICAGO	60615
A	3327	CHICAGO MEDICAL SCHOOL		3333 GREEN BAY ROAD	NORTH CHICAGO	60064
A	336	CHILDREN'S MEMORIAL HOSPITAL		2300 CHILDREN'S PLAZA	CHICAGO	60614
A	3341	COOK CO. GRAD. SCHOOL OF MED.		707 S. WOOD ST.	CHICAGO	60612
A	3383	DE PAUL UNIVERSITY		1036 W. BELDEN AVE.	CHICAGO	60614
A	3364	EUREKA COLLEGE		300 E. COLLEGE	EUREKA	61530
A	3313	EVANSTON HOSPITAL		2650 RIDGE AVE.	EVANSTON	60201
A	3328	G.D. SEARLE & CO.		BOX 5110	CHICAGO	60680
A	3331	GENERAL FOODS CORP		C/O GAINES RES. KENNELS RR 3	ST. ANNE	60964
A	3336	HEKTOEN INST./COOK CO. HOSP.		627 S. WOOD ST.	CHICAGO	60612
A	338	IIT RESEARCH INSTITUTE		10 WEST 35 ST.	CHICAGO	60616
A	3356	IL. STATE PSYCHIATRIC INST.		1601 W. TAYLOR ST.	CHICAGO 6061	62302
A	3345	ILLINOIS INST. OF TECHNOLOGY		3300 S. FEDERAL ST.	CHICAGO	60616
A	333	ILLINOIS STATE UNIVERSITY			NORMAL	61761
A	3353	ILLINOIS WESLEYAN UNIV.			BLOOMINGTON	61701
A	3343	KENDALL RESEARCH CENTER		411 LAKE ZURICH ROAD	BARRINGTON	60010
A	3389	KNOX COLLEGE			GALESBURG	61401
A	3344	KRAFT INC.		801 WAUKEGAN RD.	GLENVIEW	60025
A	3349	LAKE FOREST COLLEGE			LAKE FOREST	60046
A	3384	LARABIDA UNIV OF CHICAGO INST		5801 SOUTH ELLIS AVE.	CHICAGO	60637
A	3324	LOYOLA UNIVERSITY		1400 S. FIRST AVE.	HINES	60141
A	3367	MEDI-PHYSICS, INC.		3350 NORTH RIDGE AVE.	ARLINGTON HEIGHTS	60004
A	3377	MEMORIAL HOSPITAL		4501 NORTH PARK DR.	BELLEVIEW	62221
A	3347	MERCY HOSPITAL & MEDICAL CTR.		STEVENSON EXPRESS. AT KING DR.	CHICAGO	60616
A	338	MICHAEL REESE HOSPITAL & MEDICAL CENTER		29 & ELLIS	CHICAGO	[illegible]0616
A	3326	MOUNT SINAI HOSPITAL MEDICAL CENTER		CALIFORNIA AVE. AT 15TH ST.	CHICAGO	60608
A	3379	NORTHEASTERN ILLINOIS UNIV.		BRYN MAWR AT ST. LOUIS	CHICAGO	60625
A	3387	NORTHERN ILLINOIS UNIVERSITY			DEKALB	60115
A	3340	NORTHWESTERN UNIVERSITY		619 CLARK ST.	EVANSTON	60201
A	3394	PHARMACHEM TESTING SEVICES		17501 WEST DUVAN DR.	TINLEY PARK	60477
A	3322	PRESBYTERIAN-ST. LUKE'S HOSP.		1753 W. CONGRESS PARKWAY	CHICAGO	60612
A	3381	QUAKER OATS CO.		617 W. MAIN ST.	BARRINGTON	[illegible]10
A	3354	QUINCY COLLEGE			QUINCY	[illegible]1
A	3361	SANGAMON STATE UNIVERSITY			SPRINGFIELD	[illegible]3
X	3363	SHERMAN HOSPITAL		934 CENTER ST.	ELGIN	[illegible]20
A	3395	SHRINERS HOSP. FOR CRIPPLED CHILDREN		2211 N. OAK PARK AVE.	CHICAGO	60635
A	3375	SLEEPY HOLLOW KENNEL/CATTERY		ROUTE 2, BOX 73	MUNDELEIN	60060
A	3314	SOUTHERN ILLINOIS UNIV.			CARBONDALE	[illegible]901
A	3368	SOUTHERN ILLINOIS UNIVERSITY AT EDWARDSVILLE			EDWARDSVILLE	[illegible]025
A	3338	ST. FRANCIS HOSPITAL		355 RIDGE AVE.	EVANSTON	60202
A	3348	ST. FRANCIS HOSPITAL/MEDICAL CENTER		530 NE GLEN OAK AVE.	PEORIA	61603
A	3393	TECHNAM, INC.		2405 BOND STREET	PARK FOREST SOUTH	[illegible]337
A	3390	TOX MONITOR LAB. INC.		33 WEST CHICAGO AVENUE	OAK PARK	[illegible]302
A	3391	TOXIGENICS INC.		1800 EAST PERSHING RD.	DECATUR	62525
A	3323	TRAVENOL LABORATORIES, INC.		6301 LINCOLN AVE.	MORTON GROVE	60053
A	3336	UNIV OF IL AT CHICAGO CIRCLE		2533 UNIVERSITY HALL	CHICAGO	60680
A	3330	UNIVERSITY OF CHICAGO		5801 S. ELLIS AVE.	CHICAGO	60637
A	3318	UNIVERSITY OF ILLINOIS		833 S. WOOD ST.	CHICAGO	60612
A	3329	UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN			URBANA	61801
A	3362	WESTERN ILLINOIS UNIVERSITY			MACOMB	61456
A	3388	WHEATON COLLEGE			WHEATON	60187

INDIANA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	3221	AMERICAN MONITOR, INC.		5425 W. 94TH ST.	INDIANAPOLIS	46258
A	3211	BALL STATE UNIVERSITY			MUNCIE	47306
A	3210	BUTLER UNIVERSITY		4600 SUNSET	INDIANAPOLIS	46208
A	322	CENTRAL SOYA COMPANY, INC.			DECATUR	46733
X	324	EARLHAM COLLEGE			RICHMOND	47374
A	323	ELI LILLY & CO.		740 S. ALABAMA	INDIANAPOLIS	46206
X	3212	FT. WAYNE SURGICAL ASSOC. INC.		3124 E. STATE BLVD.	FT. WAYNE	46805
A	3223	IMC CHEMICAL GROUP INC.		PO BOX 207, 1331 S. FIRST ST.	TERRE HAUTE	47808
A	3219	INDIANA STATE UNIVERSITY			TERRE HAUTE	47809
A	326	INDIANA UNIVERSITY			BLOOMINGTON	47401
A	3224	LAFAYETTE PHARMACAL, INC.		526 N. EARL AVE.	LAFAYETTE	47902
A	325	MEAD, JOHNSON & COMPANY		2404 PENNSYLVANIA AVE.	EVANSVILLE	47721
A	329	METHODIST HOSP. OF INDIANA INC.		1604 N. CAPITAL AVE.	INDIANAPOLIS	46202
A	3215	MICHIGAN ROAD ANIMAL HOSP.		7720 N. MICHIGAN RD.	INDIANAPOLIS	46258
A	321	MILES LABORATORIES, INC.			ELKHART	46514
A	327	PURDUE UNIVERSITY			LAFAYETTE	47907
A	328	ROSE-HULMAN INC. OF TECH		5500 WABASH AVE.	TERRE HAUTE	47803
A	3222	ST. VINCENT HOSPITAL		2001 W. 86TH ST.	INDIANAPOLIS	46268
A	3220	TOXICOLOGY/PATHOLOGY SER. INC.		PO BOX 333	MT. VERNON	47620
A	3216	UNIVERSITY OF NOTRE DAME			NOTRE DAME	[illegible]656
A	3214	VALPARAISO UNIVERSITY			VALPARAISO	[illegible]383
A	3217	WABASH COLLEGE			CRAWFORDSVILLE	[illegible]933

IOWA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	4215	AMBICO, INC.		PO BOX M ROUTE 2	DALLAS CENTER	50063
A	4202	COL. OF OSTEOPATHIC MED & SURG		3200 GRAND AVE.	DES MOINES	50312
A	4205	DRAKE UNIVERSITY			DES MOINES	50311
A	4209	FORT DODGE LAB., INC.		800 FIRST ST. N.W.	FORT DODGE	50501
A	4203	IOWA STATE UNIVERSITY			AMES	50010
A	4211	LUTHER COLLEGE			DECORAH	52101
A	4216	MARIAN HEALTH CENTER		BOX 3168	SIOUX CITY	51102
A	4212	SALSBURY LABORATORIES		2000 ROCKFORD RD.	CHARLES CITY	50616
A	4213	ST. LUKE'S METHODIST HOSP.		1026 A AVENUE NE	CEDAR RAPIDS	52402
A	4201	SYNTEX (U.S.A.) INC.		PO BOX 863	DES MOINES	50304
A	4204	UNIVERSITY OF IOWA			IOWA CITY	52240

KANSAS

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	485	BAYVET CORP		PO BOX 390	SHAWNEE MISSION	66201
A	4820	BETHEL COLLEGE			NORTH NEWTON	67117
A	4819	CEVA LABORATORIES, INC.		10560 BARKLEY	OVERLAND PARK	66212
A	4811	DOUGLAS INDUSTRIES, INC.		3906 ROSEHILL RD.	LENEXA	66215
A	4815	EMPORIA STATE UNIVERSITY		1200 COMMERCIAL ST	EMPORIA	66801
A	4817	FORT HAYS STATE UNIV			HAYS	67601
A	4823	INTERX RESEARCH CORP/SUB. OF MERCK & CO. INC.		2201 WEST 21ST STREET	LAWRENCE	66044
A	4822	JACKSON BIOLOGICS, INC.		1101 W. 58 HIGHWAY	OLATHE	66061
A	481	KANSAS STATE UNIVERSITY			MANHATTAN	66502
A	4813	KRUCKENBERG, S.M.		2861 OREGON LANE	MANHATTAN	66502
A	484	MOBAY CHEMICAL CORP		PO BOX 1930	STILWELL	66085
A	489	PARSONS STATE HOSPITAL			PARSONS	67357
A	4818	PITTSBURG STATE UNIVERSITY		1701 SOUTH BROADWAY	PITTSBURG	66762
A	483	UNIV. OF KANSAS MED. CTR.		RAINBOW BLVD. AT 39TH ST.	KANSAS CITY	66103
A	482	UNIVERSITY OF KANSAS			LAWRENCE	66044
A	4821	WASHBURN UNIVERSITY OF TOPEKA		1700 COLLEGE	TOPEKA	66621
A	4814	WITCHITA STATE UNIVERSITY		1845 FAIRMOUNT	WICHITA	67208
	4812	BURROUGHS-WELLCOME				

KENTUCKY

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	516	ENVIRONMENT HEALTH RESEARCH & TESTING		2514 REGENCY RD. SUITE 105	LEXINGTON	40503
A	515	GOODWILL KENNELS		RR BOX 109	CYNTHIANA	41031
A	514	MOREHEAD STATE UNIVERSITY			MOREHEAD	40351
A	512	UNIVERSITY OF KENTUCKY			LEXINGTON	40506
A	511	UNIVERSITY OF LOUISVILLE		101 CHESTNUT	LOUISVILLE	40202

LOUISIANA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	721	ALTON OCHSNER MEDICAL FOUND.		1520 JEFFERSON HWY.	NEW ORLEANS	70121
A	726	GULF SOUTH RES. INSTITUTE		PO BOX 1177	NEW IBERIA	70560
A	723	LOUISIANA STATE UNIV. SYSTEM			BATON ROUGE	70803
A	7210	LOUISIANA TECH. UNIVERSITY			RUSTON	71270
A	7216	LOYLA UNIVERSITY		6363 ST. CHARLES AVE.	NEW ORLEANS	70118
A	7213	NICHOLLS STATE UNIVERSITY		BOX 2031 UNIVERSITY STA.	THIBODAUX	70301
A	729	NORTHEAST LOUISIANA UNIV.		4001 DESIARD	MONROE	71201
A	728	NW STATE UNIV. OF LA.			NATCHITOCHES	71457
A	722	TULANE UNIVERSITY			NEW ORLEANS	70118
A	727	UNIVERSITY OF SW LOUISIANA			LAFAYETTE	70501
A	7211	XAVIER UNIVERSITY			NEW ORLEANS	70125

MAINE

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	1111	ATLANTIC ANTIBODIES		P.O. BOX 1032	SCARBOROUGH	04074
A	118	BATES COLLEGE			LEWISTON	04240
A	119	BODIL SCHMIDT-NIELSEN		MT. DESERT ISLAND BIOLOGICAL L	SALSBURY COVE	04672
A	113	BOWDOIN COLLEGE			BRUNSWICK	04011
A	1112	DYNATECH DIAGNOSTICS, INC.		RR 1 INLAND FARM DR	SO WINDHAM	04082
A	112	MAINE MEDICAL CENTER		22 BRAMHALL ST	PORTLAND	04102
A	115	NASSON COLLEGE			SPRINGVALE	04083
A	1110	NEW ENGLAND COL OF OSTEOP MED		605 POOL ROAD	BIDDEFORD	04005
A	1114	PRESIDENT & TRUSTEES OF COLBY COLLEGE			WATERVILLE	04901
A	111	THE JACKSON LABORATORY			BAR HARBOR	04609
A	116	UNIVERSITY OF MAINE			ORONO	04473
A	1113	VENTREX LABS INC		217 READ ST	PORTLAND	04103

MARYLAND

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	5128	AM. TYPE CULTURE COLLECTION		12301 PARKLAWN DR.	ROCKVILLE	20852
A	5121	AMERICAN RED CROSS		9312 OLD GEORGETOWN RD.	BETHESDA	20014
A	MOR32	BIOCON, INC.		349 LOFSTRAND LANE	ROCKVILLE	20850
A	5124	BIOTECH RESEARCH LABS., INC.		12501 TWINBROOK PKWY	ROCKVILLE	20852
A	5127	BORRISTON RESEARCH LABS. INC		5050 BEECH PL.	TEMPLE HILLS	20031
A	5129	BURTON, PARSONS & CO., INC.		120 WESTHAMPTON AVE.	SEAT PLEASANT	20027
A	MOR33	CARNEGIE INST. OF WASHINGTON		115 W UNIVERSITY PARKWAY	BALTIMORE	21210

MARYLAND—Continued

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ODE
X	5123	COPPIN STATE COLLEGE			BALTIMORE	2121
A	51R36	COR BEL LABORATORIES, INC.		11 TAFT COURT	ROCKVILLE	2085
A	51R20	ESSEX COMMUNITY COLLEGE		7301 ROSSVILLE BLVD	BALTIMORE	2122
A	511	FLOW LABORATORIES, INC.		1710 CHAPMAN AVE.	ROCKVILLE	2085
A	5118	FRIENDS MED. SCI. RES CTR INC		22 BLOOMSBURY AVE	BALTIMORE	2122
A	51R34	GENEX CORPORATION		12300 WASHINGTON AVE.	ROCKVILLE	2085
A	5125	GILLETTE COMP RESEARCH INST		1413 RESEARCH BLVD.	ROCKVILLE	2085
X	5119	HARFORD COMMUNITY COLLEGE		401 THOMAS RUN ROAD	BEL AIR	2101
X	5110	HITTMAN ASSOCIATES, INC.		PO BOX 810	COLUMBIA	2104
A	5128	HYNSON, WESTCOTT DUNNING INC.		CHARLES & CHASE STS.	BALTIMORE	2120
A	5130	INST FOR BEHAVORAL RES. INC.		2429 LINDEN LANE	SILVER SPRING	2091
A	516	JOHN HOPKINS UNIVERSITY		34TH & CHARLES ST.	BALTIMORE	2121
A	5122	LITTON BIONETICS, INC.		5518 NICHOLSON LN	KENSINGTON	2079
A	51R38	M. A. BIOPRODUCTS		BLDG 100 BIGGS FORD ROAD	WALKERSVILLE	2179
A	5116	MD. PSYCHIATRIC RESEARCH CTR.		PO BOX 3235	BALTIMORE	2122
A	513	MICROBIOLOGICAL ASSOC., INC.		5221 RIVER ROAD	BETHESDA	2001
X	5117	PENINSULA GENERAL HOSPITAL		S. DIVISION & W. LOCUST STS.	SALISBURY	2180
A	5115	PHARMACOPATHICS RES. LAB. INC		1291 N. WASHINGTON BLVD.	LAUREL	2081
X	517	SACRED HEART HOSPITAL		900 SETON DR.	CUMBERLAND	2150
A	5111	SINAI HOSPITAL OF BALTIMORE		BELVEDERE & GREENSPRING AVES.	BALTIMORE	2121
A	5112	ST. JOSEPH HOSPITAL		7620 YORK RD.	BALTIMORE	2120
A	MDR27	THE UNION MEMORIAL HOSPITAL		201 EAST UNIVERSITY PKWY	BALTIMORE	[illegible]
X	5120	TOWSON STATE COLLEGE			BALTIMORE	2120
A	5114	UNIVERSITY OF MARYLAND		C/O DR GERALD M WARD, DIR	COLLEGE PARK	2074

MASSACHUSETTS

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	1497	APPLIED BIOMEDICAL CO.	ABIOMED	33 CC CHERRY HILL DRIVE	DENVER	0192
A	145	ARTHUR D. LITTLE, INC.		25 ACORN PARK	CAMBRIDGE	0214
A	141	ASTRA PHARMACEUTICAL PROD INC		7 NEPONSET ST.	WORCESTER	0160
A	1428	AVCO EVERETT RESEARCH LAB.		2385 REVERE BEACH PKWY.	EVERETT	0214
A	1473	BECKER JUNIOR COLLEGE		1003 MAIN ST.	LEICESTER	0152
A	1415	BETH ISRAEL HOSPITAL		330 BROOKLINE AVE.	BOSTON	0221
A	1454	BIO-RESEARCH INSTITUTE, INC.		9 COMMERCIAL AVE.	CAMBRIDGE	[illegible]
A	1483	BIOASSAY SYSTEMS CORP		225 WILDWOOD AVENUE	WOBURN	[illegible]
A	1495	BIOMEASURE INCORPORATED		11-15 E AVE.	HOPKINTON	[illegible]
A	1488	BIOTEK, INC.		21-C OLYMPIA AVE.	WOBURN	[illegible]
A	1489	BOSTON BIOMEDICAL RES. INST. INC.		20 STANIFORD ST.	BOSTON	[illegible]
A	1433	BOSTON CITY HOSPITAL		818 HARRISON AVE.	BOSTON	[illegible]
A	1460	BOSTON COLLEGE		140 COMMONWEALTH AVE.	CHESTNUT HILL	[illegible]
A	1417	BOSTON UNIVERSITY GRAD. SCHOOL		705 COMMONWEALTH AVE.	BOSTON	[illegible]
A	1430	BRANDEIS UNIVERSITY			WALTHAM	[illegible]
A	1492	BRIGHAM AND WOMEN'S HOSPITAL		75 FRANCIS STREET	BOSTON	[illegible]
A	1493	CAMBRIDGE DIAGNOSTICS, INC.		358 ALLSTON STREET	CAMBRIDGE	[illegible]
A	1469	CAMBRIDGE NUCLEAR RADIOPHARMACEUTICAL CENTER		575 MIDDLESEX TURNPIKE	BILLERICA	[illegible]
A	1420	CHILDREN'S HOSP. MEDICAL CTR.		300 LONGWOOD AVE.	BOSTON	[illegible]
A	1429	CLARK UNIVERSITY		950 MAIN ST.	WORCESTER	[illegible]
A	1475	COLLABORATIVE RESEARCH, INC		1365 MAIN ST	WALTHAM	[illegible]
A	1496	DR. OVID FRASER		79 MARLBOROUGH RD. BOX 215	SOUTHBOROUGH	[illegible]
A	1496	DR. OVID FRASER		79 MARLBOROUGH RD. BOX 215	SOUTHBOROUGH	[illegible]
A	146	EG&G MASON RESEARCH INSTITUTE		57 UNION ST.	WORCESTER	[illegible]
A	1410	EYE RESEARCH INST./RETINA FOUNDATION		20 STANIFORD ST.	BOSTON	[illegible]
A	1480	FINDLEY RESEARCH INC.		PO BOX 375	ASSONET	[illegible]
A	1437	FORSYTH DENTAL CENTER		140 THE FENWAY	BOSTON	[illegible]
A	1478	GAMMA DIAGNOSTIC LABS., INC.		50 WALTON ST.	ATTLEBORO	[illegible]
A	1472	GTE LABORATORIES, INC.		40 SYLVAN ROAD	WALTHAM	[illegible]
A	1419	HARVARD MEDICAL SCHOOL		25 SHATTUCK ST.	BOSTON	[illegible]
A	1425	HOLYOKE COMMUNITY COLLEGE		303 HOMESTEAD AVE.	HOLYOKE	[illegible]
A	1471	JOSLIN DIABETES FOUND., INC.		1 JOSLIN PL.	BOSTON	[illegible]
A	1416	LAHEY CLINIC FOUNDATION		41 BURLINGTON MALL RD.	BURLINGTON	[illegible]
A	144	LEMUEL SHATTUCK HOSPITAL		170 MORTON ST.	JAMAICA PLAIN	[illegible]
A	1487	LIBERTY MUTUAL RESEARCH CT		71 FRANKLAND RD	HOPKINTON	[illegible]
A	147	MASS. COLLEGE OF PHARMACY & ALLIED HEALTH SCIENCES		179 LONGWOOD AVE.	BOSTON	[illegible]
A	1422	MASS. EYE & EAR INFIRMARY		243 CHARLES ST.	BOSTON	[illegible]
A	1418	MASS. INSTITUTE OF TECHNOLOGY		77 MASSACHUSETTS AVE.	CAMBRIDGE	[illegible]
A	1414	MASSACHUSETTS GENERAL HOSP			BOSTON	[illegible]
A	1498	MCLEAN HOSPITAL CORPORATION		115 MILL STREET	BELMONT	[illegible]
A	1464	MILLIPORE CORPORATION		ASHBY ROAD	BEDFORD	[illegible]
A	1490	MOUNT IDA JUNIOR COLLEGE		777 DEDHAM STREET	NEWTON CENTER	[illegible]
A	1424	MT. HOLYOKE COLLEGE			SOUTH HADLEY	[illegible]
A	1444	NEW ENGLAND DEACONESS HOSP		185 PILGRIM RD.	BOSTON	[illegible]
A	1423	NEW ENGLAND NUCLEAR CORPORATION		501 TREBLE COVE RD	NORTH BILLERICA	[illegible]
A	1474	NEWBURY JUNIOR COLLEGE		100 SUMMER ST.	HOLLISTON	[illegible]
A	1421	NORTHEASTERN UNIVERSITY		360 HUNTINGTON AVE.	BOSTON	[illegible]
A	1432	SHRINERS BURNS INSTITUTE		50 BLOSSOM ST.	BOSTON	[illegible]
A	1479	SHRIVER CTR., MENT RETARD INC.		200 TRAPELO ROAD	WALTHAM	[illegible]
A	143	SIDNEY FARBER CANCER INSTITUTE		44 BINNEY ST.	BOSTON	[illegible]
A	1466	SIMMONS COLLEGE	PARK SCIENCE CENTER	300 THE FENWAY	BOSTON	[illegible]
A	1461	SMITH COLLEGE			NORTHAMPTON	[illegible]
A	1413	ST. ELIZABETH'S HOSPITAL		736 CAMBRIDGE ST.	BRIGHTON	[illegible]
A	1461	ST. MARGARET'S HOSP FOR WOMEN		90 CUSHING AVE.	DORCHESTER	[illegible]
A	1411	ST. VINCENT HOSPITAL		25 WINTHROP ST.	WORCESTER	[illegible]
A	1463	STATE LABORATORY INSTITUTE/MASS. DEPT. OF PUBLIC HEALTH		305 SOUTH ST.	JAMAICA PLAIN	[illegible]
A	1439	STONEHILL COLLEGE			NORTH EASTON	[illegible]
A	1477	THE MEMORIAL HOSPITAL		119 BELMONT ST.	WORCESTER	[illegible]
A	148	TOXICOL-SISA, INC.		1530 CONCORD AVE.	CAMBRIDGE	[illegible]
A	1446	TRUSTEES OF HAMPSHIRE COLLEGE			AMHERST	[illegible]
A	1484	TUFTS UNIVERSITY		BALLOU HALL	MEDFORD	[illegible]
A	1482	TUFTS-NEW ENG MED CTR., INC		BOX 237-171 HARRISON AVE	BOSTON	[illegible]

MASSACHUSETTS—Continued

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	1461	UNIVERSITY OF LOWELL		450 AIKEN ST	LOWELL	01854
A	1435	UNIVERSITY OF MASSACHUSETTS			AMHERST	01002
A	1494	WELLESLEY COLLEGE		WELLESLEY COLLEGE SCIENCE CNTR	WELLESLEY	02181
A	1438	WILLIAMS COLLEGE			WILLIAMSTOWN	01267
A	1468	WORCESTER POLYTECHNIC INST		WEST ST	WORCESTER	01609
A	1412	WORCHESTER FOUND FOR EXP BIO		222 MAPLE AVE	SHREWSBURY	01545

MICHIGAN

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	3420	ALBION COLLEGE			ALBION	49224
A	347	BLODGETT MEMORIAL HOSPITAL		1840 WEALTHY, S.E.	GRAND RAPIDS	49506
A	3448	C.S. MOTT COMMUNITY COLLEGE		1401 E. COURT ST	FLINT	48503
A	3444	CENTRAL MICHIGAN UNIVERSITY			MT. PLEASANT	48858
A	3436	CHILDREN'S HOSP OF MICHIGAN		3901 BEAUBIEN	DETROIT	48201
A	3457	DAVENPORT COLLEGE OF BUSINESS	CENTER FOR THE STUDY OF E M S	415 EAST FULTON	GRAND RAPIDS	49503
A	3446	DELTA COLLEGE		UNIVERSITY CENTER, R STA.	BAY CITY	48710
A	3428	DETROIT-MACOMB HOSP ASSN.		7815 E. JEFFERSON	DETROIT	48214
A	3449	DORNER LABORATORIES		3203 W. COLUMBIA	BATTLE CREEK	49017
A	348	DOW CHEMICAL CO.			MIDLAND	48640
A	3432	DOW CORNING CORPORATION		SOUTH SAGINAW RD	MIDLAND	48640
A	3419	FERRIS STATE COLLEGE			BIG RAPIDS	49307
A	3450	FOUNDATION FOR BEHAVIORAL RESEARCH		BOX 248, 600 CHERRY ST.	AUGUSTA	49012
A	3452	GENERAL MOTORS RESEARCH LABS.		12 MILE & MOUND RDS.	WARREN	48090
A	3440	GRAND VALLEY STATE COLLEGE		COLLEGE LANDING	ALLENDALE	49401
A	3410	HENRY FORD HOSPITAL & EDSEL B. FORD INSTITUTE FOR MEDICA		2799 W. GRAND BLVD.	DETROIT	48202
A	3435	HOPE COLLEGE			HOLLAND	49423
A	3447	HUTZEL HOSPITAL		432 E. HANCOCK	DETROIT	48201
A	3416	INGHAM MEDICAL HOSPITAL		401 W. GREENLAWN	LANSING	48910
A	343	INTERNATIONAL RES. & DEV CORP		300 MAIN ST.	MATTAWAN	49071
A	3441	JAFFEE, MARTIN D.		2110-16TH ST.	BAY CITY	48706
A	3458	KERN HOSPITAL		21230 DEQUINDRE	WARREN	48091
A	3434	LABORATORY RES. ENTERP INC.		5251 SOUTH 6TH ST.	KALAMAZOO	49001
A	3411	LAFAYETTE CLINIC		951 E. LAFAYETTE	DETROIT	48207
A	3443	LAKE SUPERIOR STATE COLLEGE			SAULT STE. MARIE	49783
A	3466	LOWE, HENRY E	RESEARCH & PRODUCT DEVELOPMENT CT	21725 ALLEGHENY STREET	CASSOPOLIS	49031
A	3453	MARQUETTE GENERAL HOSPITAL			MARQUETTE	49855
A	3451	MCLAREN GENERAL HOSPITAL		401 S. BALLENGER HWY.	FLINT	48502
A	3427	MI. DEPT OF PUBLIC HEALTH		3500 N. LOGAN	LANSING	48914
A	3439	MICHIGAN CANCER FOUNDATION		110 EAST WARREN AVE.	DETROIT	48201
A	3417	MICHIGAN STATE UNIVERSITY			EAST LANSING	48823
A	3442	MICHIGAN TECHNOLOGICAL UNIV.			HOUGHTON	49931
A	3412	MT. CARMEL MERCY HOSPITAL		6071 W. OUTER DRIVE	DETROIT	48235
A	3421	NORTHERN MICHIGAN UNIVERSITY			MARQUETTE	49855
A	3438	OAKLAND UNIVERSITY			ROCHESTER	48063
A	349	PROVIDENCE HOSPITAL		16001 NINE MILE RD.	SOUTHFIELD	48075
A	348	SINAI HOSPITAL OF DETROIT		6767 W. OUTER DR.	DETROIT	48235
A	3454	TOXICITY RESEARCH LAB.		510 WEST HACKLEY	MUSKEGON	49444
A	3424	UNIVERSITY OF DETROIT		4001 W. MCNICHOLS RD.	DETROIT	48221
A	341	UNIVERSITY OF MICHIGAN			ANN ARBOR	48104
A	3415	UPJOHN COMPANY		301 HENRIETTA ST.	KALAMAZOO	49001
A	345	WARNER-LAMBERT/PARK-DAVIS		GPO BOX 1047	ANN ARBOR	48106
A	3418	WAYNE COUNTY GENERAL HOSPITAL			ELOISE	48132
A	3414	WAYNE STATE UNIVERSITY			DETROIT	48202
A	3433	WESTERN MICHIGAN UNIVERSITY			KALAMAZOO	49001
A	3437	WILLIAM BEAUMONT HOSPITAL		3601 W. 13 MILE RD.	ROYAL OAK	48073

MINNESOTA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	4121	APPLIED CARDIOPULMONARY RESEARCH FOUNDATION		2545 CHICAGO AVE SUITE 111	MINNEAPOLIS	55404
A	4110	AUSTIN COMMUNITY COLLEGE		1600-8TH AVE., N.W.	AUSTIN	55912
A	4122	CARDIAC PACEMAKERS, INC		4100 N HAMLINE AVE	ST PAUL	55108
A	4123	DAIG CORPORATION		14901 INDUSTRIAL RD	MINNETONKA	55343
A	4119	KALLESTAD LABORATORIES		1000 LAKE HAZELTINE DR.	CHASKA	55318
A	4114	MANKATO STATE UNIVERSITY			MANKATO	56001
A	418	MAYO FOUNDATION		200 FIRST ST. S.W.	ROCHESTER	55901
A	4115	MEDTRONIC, INC.		1500 NORTHDALE BLVD NW	MINNEAPOLIS	55433
A	417	MINNEAPOLIS MED RES FOUND INC.		619 S. 5TH ST.	MINNEAPOLIS	55415
A	4118	NORTHWESTERN HOSPITAL		810 E. 27TH ST.	MINNEAPOLIS	55407
A	416	RIKER LAB. INC./SUBSIDIARY 3M CO.		3M CENTER, BLDG. 218-3	ST PAUL	55101
A	4124	RIVER VALLEY FARMS		BOX 4	MARINE ON ST. CROIX	55047
A	4112	SOUTHWEST STATE UNIVERSITY			MARSHALL	56258
A	4111	ST. JOHN'S UNIVERSITY			COLLEGEVILLE	56321
A	413	ST. PAUL-RAMSEY HOSPITAL		640 JACKSON ST.	ST. PAUL	55101
A	415	UNIVERSITY OF MINNESOTA			MINNEAPOLIS	55455

MISSISSIPPI

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	562	MISSISSIPPI STATE UNIVERSITY		DRAWER G	STATE COLLEGE	39762
A	561	UNIVERSITY OF MISSISSIPPI		OFFICE OF THE CHANCELLOR	UNIVERSITY	38677
A	564	UNIVERSITY OF SOUTHERN MISSISSIPPI		SOUTHERN STATION	HATTIESBURG	39401

MISSOURI

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	4310	CURATORS OF THE UNIV. OF MO.			COLUMBIA	01
A	4335	FARMLAND INDUSTRIES, INC.		P.O. BOX 7305	KANSAS CITY	116
A	431	INST. OF MED. EDUCATION & RES		1606 S. 14TH ST.	ST. LOUIS	63104
A	437	JEWISH HOSPITAL OF ST. LOUIS		216 S. KINGSHIGHWAY BLVD.	ST. LOUIS	63110
A	4331	JR COLL. DIST OF JEFFERSON CO			HILLSBORO	63050
A	4321	KANSAS CITY COLL OSTEOP MED		2105 INDEPENDENCE BLVD.	KANSAS CITY	64124
A	4312	KIRKSVILLE COLL OSTEOP & SURG			KIRKSVILLE	63501
A	4332	LOGAN COLLEGE OF CHIROPRACTIC		1851 SCHOETTLER RD.	CHESTERFIELD	63017
A	435	MALLINCKRODT CHEMICAL WORKS		2ND & MALLINCKRODT STS.	ST. LOUIS	63150
A	4329	MAPLE WOODS COMMUNITY COLLEGE		2601 NE BARRY RD.	KANSAS CITY	64156
A	4324	MARION LABORATORIES, INC.		10236 BUNKER RIDGE RD.	KANSAS CITY	64137
A	439	MIDWEST RESEARCH INSTITUTE		425 VOLKER BLVD.	KANSAS CITY	64114
A	4323	MISSOURI ANALYTICAL LAB. INC.		1620 DELMAR BOULEVARDS	ST. LOUIS	63103
A	4333	MONSANTO COMPANY		800 N LINDBERGH BLVD	ST. LOUIS	63166
A	4330	NORTHEAST MISSOURI STATE UNIV.			KIRKSVILLE	63501
A	4314	PHILIPS ROXANE, INC.		2621 N. BELT HIGHWAY	ST. JOSEPH	64502
A	436	RALSTON PURINA CO.		CHECKERBOARD SQUARE	ST. LOUIS	63188
A	432	SCIENTIFIC ASSOCIATES, INC.		6200 S. LINDBERGH	ST. LOUIS	63123
A	4325	SIGMA INTERNATIONAL, LTD.		3500 DEKALB ST.	ST. LOUIS	63129
A	4315	ST. JOHN'S MERCY HOSPITAL		621 S. NEW BALLAS ROAD	ST. LOUIS	63141
A	433	ST. LOUIS COLLEGE OF PHARMACY		4588 PARKVIEW PLACE	ST. LOUIS	63110
A	4311	ST. LOUIS UNIVERSITY		1402 S. GRAND AVE.	ST. LOUIS	63104
X	4327	ST. MARY'S HOSPITAL		101 MEMORIAL DR.	KANSAS CITY	64108
A	438	WASHINGTON UNIVERSITY		LINDELL & SKINKER BLVDS.	ST. LOUIS	63130
A	4322	WILLIAM JEWELL COLLEGE			LIBERTY	64068
A	4326	YOUNGER LABORATORIES, INC.		123 CLIFF CAVE RD.	ST. LOUIS	63129

MONTANA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	612	MONTANA STATE UNIVERSITY			BOZEMAN	59715
A	614	RIBI IMMUNOCHEM RESEARCH, INC.		P.O. BOX 1409	HAMILTON	59840
A	611	UNIVERSITY OF MONTANA			MISSOULA	59801

NEBRASKA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	4710	BURNS-BIOTEC LABS INC		220 & WEST CENTER RD.	ELKHORN	68022
X	4712	CENTRAL BIOLOGICAL CO.		3836 J ST.	OMAHA	68127
A	4702	CREIGHTON UNIV/SCHOOL OF MED.		529 N. 27TH ST	OMAHA	681[illegible]1
A	4708	DANA COLLEGE			BLAIR	[illegible]
A	4705	DELLEN, INC.		77TH & SERUM AVE.	RALSTON	[illegible]7
A	4709	FATHER FLANAGAN'S BOYS' HOME			BOYS TOWN	[illegible]0
X	4703	HARRIS LABORATORIES, INC.		PO BOX 427, 624 PEACH ST.	LINCOLN	[illegible]01
A	4711	MIDLAND LUTHERAN COLLEGE		720 E. 9TH	FREMONT	68025
A	4707	NEBRASKA WESLEYAN UNIVERSITY		50TH & ST. PAUL AVE.	LINCOLN	68504
A	4713	OMAHA COLLEGE OF HEALTH CAREERS		1052 PARK AVE.	OMAHA	68105
A	4704	UNIVERSITY OF NEBRASKA		3835 HOLDREGE	LINCOLN	68503

NEVADA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	881	UNIVERSITY OF NEVEDA			RENO	89507

NEW HAMPSHIRE

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	121	DARTMOUTH COLLEGE		PO BOX 432	HANOVER	03755
A	126	IMMUNOGENETICS, INC.		50 CHESTNUT ST.	DOVER	03820
A	122	KEENE STATE COLLEGE			KEENE	03431
A	123	UNIVERSITY OF NEW HAMPSHIRE		SPAULDING BLDG.	DURHAM	03824

NEW JERSEY

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	2274	ALLIED CHEMICAL CORP		PO BOX 1057R	MORRISTOWN	07960
A	2213	AMR BIOLOGICAL RESEARCH INC.		880-892 SOUTH CLINTON AVE.	TRENTON	08611
A	2241	BECTON, DICKINSON & COMPANY		MACK CENTRE DRIVE	PARAMUS	07652
A	2280	BERLEX LABORATORIES INC		110 E HANOVER AVE	CEDAR KNOLLS	07927
A	2283	BIO-SAFETY RESEARCH LABS.		MILK & BROAD STS.	BRANCHVILLE	07826
A	2233	BIO/DYNAMICS INC		METTLERS RD.	EAST MILLSTONE	08873
A	2281	BIOLOGICAL CORP OF AMERICA		40 MARKLEY ST	PORT READING	07064
A	2283	BLOOMFIELD COLLEGE	DEPT. OF BEHAV. BIOLOGY/ PHARMACOLOGY		BLOOMFIELD	07003
A	2221	BRISTOL-MYERS PRODUCTS		225 LONG AVE.	HILLSIDE	07205
A	2276	CAMDEN COUNTY COLLEGE		PO BOX 200	BLACKWOOD	08012
A	2214	CIBA PHARMACEUTICAL CO.		556 MORRIS AVE.	SUMMIT	07901
A	2280	CLINICAL SCIENCES, INC.		30 TROY RD.	WHIPPANY	07981
A	2288	COLL. OF MED & DENISTRY OF NJ		PO BOX 101	PISCATAWAY	08854
A	2220	COLLEGE OF MEDICINE & DENTISTRY OF N. J.		100 BERGEN ST.	NEWARK	07103
A	2289	CONSUMER PROD. TESTING CO. INC		1275 BLOOMFIELD AVE. BLDG. #2-	FAIRFIELD	6
A	2273	COSMOPOLITAN SAFETY EVALUATION, INC.		MUD CUT RD. & RT. 15	LAFAYETTE	48
A	2272	COUNTY COLLEGE OF MORRIS		RT. #10 & CENTER GROVE RD	DOVER	07801

NEW JERSEY—Continued

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	2210	CYANAMID FNDN. FOR AGRI. DEV.		PO BOX 400	PRINCETON	08540
A	2212	ETHICON RESEARCH FOUNDATION		U.S. HIGHWAY 22	SOMERVILLE	08876
A	2229	FAIRLEIGH DICKINSON UNIV.		1000 RIVER ROAD	TEANECK	07666
A	2288	FMC CORP/ENVIRONMENTAL & HEALTH LAB.		78 FOURTH ST	SOMERVILLE	08876
A	2258	FOOD & DRUG RES. LAB., INC.		90 EVERGREEN PLACE	EAST ORANGE	07019
A	2290	GENERAL FOODS CORPORATION		250 NORTH STREET	WHITE PLAINS	10625
A	2275	GIBRALTAR BIO LAB. INC		23 JUST RD	FAIRFIELD	07006
A	2277	HILLSIDE ACRES INC		GREEN POND RD BOX 52	BUTTZVILLE	07829
A	2242	HOECHST-ROUSSEL PHARMACEUT		RT. 202-206 NORTH	SOMERVILLE	08876
A	2232	HOFFMAN-LA ROCHE, INC.		340 KINGSLAND ST.	NUTLEY	07110
A	2237	INSTITUTE FOR MEDICAL RES.		COPEWOOD ST	CAMDEN	08103
A	2287	JOHNSON & JOHNSON BABY PRODUCTS COMPANY		220 CENTENNIAL AVE	PISCATAWAY	08854
A	2216	JOHNSON & JOHNSON RES. FNDN.		ROUTE 1	N. BRUNSWICK	08903
A	224	LEBCRCO LABORATORIES		123 HAWTHORNE ST	ST ROSELLE PARK	07204
A	2271	LEVER BROTHERS COMPANY		45 RIVER RD.	EDGEWATER	07020
X	2240	MANZIANO, DR. CLARENCE F		903 WESTSIDE AVE.	JERSEY CITY	07304
A	2230	MERCK & CO., INC.		126 E. LINCOLN AVE.	RAHWAY	07065
X	2235	MONMOUTH MEDICAL CENTER		3RD & PAVILION AVE.	LONG BRANCH	07740
A	225	N.J. STATE DEPT. OF HEALTH		BOX 1540	TRENTON	08625
A	2298	NABISCO, INC.		RIVER ROAD	EAST HANOVER	07936
A	2231	NEWARK BETH ISRAEL HOSPITAL		201 LYONS AVE.	NEWARK	07112
A	2282	NUTRITION INTERNATIONAL	PRODUCT SAFETYLABS	725 CRANBURY ROAD	EAST BRUNSWICK	08816
A	2284	ORTHO DIAGNOSTIC SYSTEMS INC.		ROUTE 202	RARITAN	08869
A	226	ORTHO RESEARCH INSTITUTE		U.S. HIGHWAY 202	RARITAN	08869
A	2278	PHARMACIA INC		800 CENTENNIAL AVE	PISCATAWAY	08854
A	2244	PITMAN-MOORE, INC.		PO BOX 344	WASHINGTON CROSSING	08560
A	2249	REED & CARNRICK		30 BORIGHT AVE.	KENILWORTH	07033
A	2225	RUTGERS, THE STATE UNIVERSITY			NEW BRUNSWICK	08903
A	2248	S. MOUNTAIN LIFE SCIENCES, INC.		1102 INDUSTRIAL PKWY.	BRICKTOWN	08723
A	229	SANDOZ PHARMACEUTICALS			HANOVER	07936
A	2236	SCHERING CORP		60 ORANGE ST.	BLOOMFIELD	07003
A	2246	SETON HALL UNIVERSITY			SOUTH ORANGE	07079
A	2226	SOUTH MOUNTAIN LABS, INC.		380 LACKAWANNA PL.	S ORANGE	07079
A	2228	SQUIBB AGRI. RESEARCH CNTR		3 BRIDGES RD.	THREE BRIDGES	08887
A	2215	ST. BARNABAS MEDICAL CENTER		OLD SHORT HILLS ROAD	LIVINGSTON	07039
X	227	ST. MICHAEL'S HOSPITAL		306 HIGH ST.	NEWARK	07102
A	2222	TRUSTEES OF PRINCETON UNIV.		NEW SOUTH BLDG.	PRINCETON	08540
A	2250	U.S. TESTING CO., INC.		1415 PARK AVE.	HOBOKEN	07030
A	2239	UNIVERSITY LABORATORIES, INC.		810 N. SECOND AVE.	HIGHLAND PARK	08904
A	222	WARNER-LAMBERT RESEARCH INST.		170 TABOR RD.	MORRIS PLAINS	07950
A	2211	WELLS LABS., INC.		25-27 LEWIS AVE.	JERSEY CITY	07306
A	2285	WILLIAM PATERSON COLLEGE		300 POMPTON RD.	WAYNE	07470
A	2286	WOOD, SUZANNE		5 E LAKE CIRCLE DRIVE	MARLTON	08053

NEW MEXICO

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	8611	COULSTON INTERNATIONAL, INC.		2512 CHRISTINA PLACE	ALAMOGORDO	88310
A	868	EASTERN NEW MEXICO UNIVERSITY			PORTALES	88130
A	8610	GRUENERWALD, W		1020 HOLLY SUGAR BLDG.	COLORADO SPRINGS	80903
A	861	LOS ALAMOS SCIENTIFIC LAB.		PO BOX 1663	LOS ALAMOS	87545
A	863	LOVELACE BIOMEDICAL & ENVIROMENTAL RES. INST.		P.O. BOX 5890	ALBUQUERQUE	87185
A	8612	LOVELACE MEDICAL FOUNDATION		5400 GIBSON BLVD., SE	ALBUQUERQUE	87108
A	867	N.M. INST. OF MINING & TECH.			SOCORRO	87801
A	859	REGENTS OF NM STATE UNIV.		BOX 3699	LAS CRUCES	88003
A	862	UNIVERSITY OF NEW MEXICO			ALBUQUERQUE	87106
A	8613	ZIA INSTITUTE FOR ANIMAL RES., INC.		5800 DELMAR AVE., N.E.	ALBUQUERQUE	87111

NEW YORK

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	21131	AGWAY RESEARCH LABORATORY		777 WARREN RD.	ITHACA	14850
A	2169	ALBANY COLLEGE OF PHARMACY		106 N. SCOTLAND AVE.	ALBANY	12208
A	21103	ALBANY MEDICAL COLLEGE		47 NEW SCOTLAND AVE.	ALBANY	12208
A	2163	ALFRED UNIVERSITY			ALFRED	14802
A	21106	AM STANDARDS TESTING BUR. INC		40 WATER ST.	NEW YORK	10004
A	2154	AM. MUSEUM OF NATURAL HISTORY		CENTRAL PARK WEST AT 79 ST.	NEW YORK	10024
A	2124	AMERICAN CYANAMID CO.		N. MIDDLETOWN RD.	PEARL RIVER	10965
A	21148	AMERICAN HEALTH FOUNDATION		320 EAST 43RD ST.	NEW YORK	10017
X	2194	ANIMAL MEDICAL CENTER		510 E. 62ND ST.	NEW YORK	10021
A	2139	ARNOT-OGDEN MEMORIAL HOSPITAL		ROE AVE.	ELMIRA	14901
A	2115	ASSOCIATED UNIVERSITIES, INC.			UPTON	11973
A	2150	AVON PRODUCTS, INC., TOXICOLOGY DEPT.		DIVISION STREET	SUFFERN	10901
A	21138	AYERST RESEARCH LABORATORIES			CHAZY	12921
X	21152	BARBARA KOPP GERIATRIC RES CT		100 THORNTON AVE	AUBURN	13021
A	2133	BETH ISRAEL MEDICAL CENTER		10 NATHAN D. PERLMAN PL.	NEW YORK	10003
A	21166	BIOSAFE SYSTEMS, INC.		1804 PLAZA AVE.	NEW HYDE PARK	11040
A	21158	BIOSPHERE RESEARCH CENTER, INC.		216 CONGERS ROAD	NEW CITY	10956
X	21120	BOOTH MEMORIAL HOSPITAL		MAIN ST. AT BOOTH MEMORIAL AVE	FLUSHING	11355
A	2141	BOYCE THOMPSON INSTITUTE		TOWER ROAD	ITHACA	14853
A	2148	BRISTOL LABORATORIES		PO BOX 657	SYRACUSE	13201
A	2146	BRONX-LEBANON HOSPITAL CENTER		1276 FULTON AVE.	BRONX	10456
A	2158	BROOKDALE HOSPITAL CENTER		BROOKDALE PLAZA	BROOKLYN	11212
A	21116	BUFFALO GENERAL HOSPITAL		100 HIGH ST	BUFFALO	14203
X	21150	BUFFALO TESTING LABS., INC.		902 KENMORE AVENUE	BUFFALO	14216
A	21134	BUREAU OF LABS., CITY OF NY		455 FIRST AVENUE	NEW YORK	10016
A	2149	CANISIUS COLLEGE		2001 MAIN ST.	BUFFALO	14208
A	21127	CARTER-WALLACE, INC.		HALF ACRE RD.	CRANBURY	08512
X	2147	CATTARAUGUS COUNTY LABORATORY		302 LAURENS ST.	OLEAN	14760
A	21162	CHARLES RIVER RESEARCH PRIMATES CORP		P.O. BOX 416	PORT WASHINGTON	11050

NEW YORK—Continued

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	21138	CHILDRENS HOSP OF BUFFALO		219 BRYANT ST.	BUFFALO	2
A	21167	CINTICHEM INC.		P.O. BOX 816	TUXEDO	987
A	21142	CITY UNIVERSITY OF N.Y.		1515 BROADWAY	NEW YORK	10036
A	21110	COLD SPRINGS HARBOR LAB.		P.O. BOX 100	COLD SPRINGS HARBOR	11724
A	212	COLGATE UNIVERSITY			HAMILTON	13346
A	21164	COLGATE-PALMOLIVE COMPANY		300 PARK AVE.	NEW YORK	10022
A	21114	CORNELL UNIV. MEDICAL COLLEGE		1300 YORK AVE.	NEW YORK	10021
A	21119	CORNELL UNIVERSITY			ITHACA	14850
X	2182	CORNING GLASS WORKS		SULLIVAN PARK	CORNING	14830
X	21161	CDH2 TOXICOLOGY RESEARCH, INC.		500 RIVER STREET	ROCHESTER	14612
X	2156	D'YOUVILLE COLLEGE		320 PORTER AVE.	BUFFALO	14201
A	2162	EASTMAN DENTAL CENTER		625 ELMWOOD AVE.	ROCHESTER	14620
A	2187	EASTMAN KODAK CO.		KODAK PARK	ROCHESTER	14650
X	2190	ELMIRA COLLEGE		COLLEGE AVENUE	ELMIRA	14901
A	21154	EM INDUSTRIES, INC		35 CHANNEL DRIVE	PORT WASHINGTON	11050
A	21115	ENDO LABORATORIES, INC.		1000 STEWART AVE.	GARDEN CITY	11530
X	2110	ERIE COMMUNITY COLLEGE		4140 S WESTERN BLVD	ORCHARD PARK	14127
A	2169	ERIE COUNTY MEDICAL CTR		462 GRIDER ST.	BUFFALO	14215
A	21148	FORDHAM UNIVERSITY		E. FORDHAM RD.	BRONX	10458
A	2173	GENESEE HOSPITAL		224 ALEXANDER ST.	ROCHESTER	14607
X	21160	GUERRA, LADISLAO A.	LAG BIOLOGICALS, INC.	725 W 172ND STREET	NEW YORK	10032
A	21125	HEALTH RESEARCH, INC.		EMPIRE STATE PLAZA TOWER BLDG.	ALBANY	12208
A	2161	HOBARTH & WM. SMITH COLLEGES		ST. CLAIR ST.	GENEVA	14456
A	2186	HOFSTRA UNIVERSITY		HEMPSTEAD TPK	HEMPSTEAD	11550
A	21166	HOSPITAL FOR JOINT DISEASES ORTHOPAEDIC INSTITUTE	BETH ISRAEL MEDICAL CENTER	301 EAST 17TH STREET	NEW YORK	10003
A	21151	HOUGHTON COLLEGE			HOUGHTON	14744
A	2183	HUDSON VALLEY COMM. COLLEGE		80 VANDENBURGH AVE.	TROY	12180
A	2184	JAMESTOWN COMMUNITY COLLEGE		525 FALCONER ST.	JAMESTOWN	14701
A	21129	JEWISH HOSP/MED CTR/BROOKLYN		555 PROSPECT PLACE	BROOKLYN	11238
A	2198	KINGSBROOK JEWISH MED. CNTR.		86 E. 49TH ST.	BROOKLYN	11203
A	2191	LENOX HILL HOSPITAL		100 E. 77TH ST.	NEW YORK	10021
A	21117	LONG ISLAND COLLEGE HOSPITAL		340 HENRY ST.	BROOKLYN	11201
A	21137	LONG ISLAND JEWISH MED. CNTR.		270-05 76 AVE.	NEW HYDE PARK	11040
A	21122	LONG ISLAND UNIVERSITY		UNIVERSITY CENTER	BROOKVILLE	11548
A	2176	MAIMONIDES MEDICAL CENTER		4802 TENTH AVE.	BROOKLYN	11219
A	21912	MARY IMOGENE BASSET HOSPITAL		ATWELL RD.	COOPERSTOWN	13326
A	2117	MASONIC MEDICAL RESEARCH LAB.		BLEECKER ST.	UTICA	13501
A	2167	MEDICAL FOUNDATION OF BUFFALO		73 HIGH ST.	BUFFALO	14203
A	2142	METHODIST HOSP OF BROOKLYN		506 SIXTH ST.	BROOKLYN	11215
A	211	MILLARD FILLMORE HOSPITAL		3 GATES CIRCLE	BUFFALO	14207
A	21153	MOBIL OIL CORP	ENVIRON. & TOXICOL. DPT	150 EAST 42 ST	NEW YORK	10017
A	2181	MONTEFIORE HOSP & MED. CNTR.		111 E. 210 ST.	BRONX	10467
A	2140	MT. SINAI SCHOOL OF MEDICINE		100 ST. & 5TH AVE.	NEW YORK	10029
A	21133	N.Y. DEPT. OF MENTAL HYGIENE		44 HOLLAND AVE.	ALBANY	12229
A	21[illegible]3	N.Y.C. HEALTH & HOSP CORP		125 WORTH ST.	NEW YORK	10013
A	2179	NACC TESTING & RESEARCH LAB.		80 HANSON PL.	BROOKLYN	7
A	2174	NASSAU COUNTY MEDICAL CTR.		2201 HEMPSTEAD TURNPIKE	EAST MEADOW	5
X	2146	NASSAU HOSPITAL		259 FIRST ST.	MINEOLA	1
A	2160	NEW YORK MEDICAL COLLEGE		FIFTH AVENUE AT 106 ST.	NEW YORK	29
A	21118	NEW YORK UNIVERSITY		WASHINGTON SQUARE	NEW YORK	10003
A	21107	NORTH SHORE HOSPITAL		300 COMMUNITY DR.	MANHASSET LONG IS.	11030
A	2122	NORWICH EATON PHARMACEUTICALS INC.	PRODUCT SAFETY LABS	PO BOX 191	NORWICH	13815
A	21124	NYS DEPARTMENT OF HEALTH		EMPIRE STATE PLAZA	ALBANY	12237
A	2168	PFIZER, INC.		235 E. 42ND ST.	NEW YORK	10017
X	2123	POLYTECHNIC INST. OF BROOKLYN		333 JAY ST.	BROOKLYN	11201
A	21135	PUBLIC HEALTH RESEARCH INST. OF NYC		455 FIRST AVENUE	NEW YORK	10016
X	21158	QUEENS CHILDREN PSYCHIATRIC CT		74-03 COMMONWEALTH BLVD	BELLEROSE	11426
A	21130	RENSSELAER POLYTECHNIC INST			TROY	12181
A	2143	REVLON RESEARCH CENTER, INC.		945 ZEREGA AVE.	BRONX	10473
A	21140	ROCHESTER GENERAL HOSPITAL		1425 PORTLAND AVE.	ROCHESTER	14621
A	21128	ROCHESTER INSTITUTE OF TECH.		1 LOMB MEMORIAL DR.	ROCHESTER	14623
A	2138	ROCKEFELLER UNIVERSITY		YORK AVE. AT 66TH ST.	NEW YORK	10021
A	2158	SCHWARTZ COLL. OF PHARMACY		75 DEKALB AVE.	BROOKLYN	11201
A	21100	SKIDMORE COLLEGE			SARATOGA SPRINGS	28661
A	2172	SLOAN-KETTERING INST/CANCER		410 E. 68TH ST.	NEW YORK	10021
X	2178	ST. BARNABAS HOSPITAL		183RD & THIRD STS.	BRONX	10457
A	2198	ST. BONAVENTURE UNIVERSITY			ST. BONAVENTURE	14778
X	2157	ST. JOHN FISHER COLLEGE		3690 EAST AVE.	ROCHESTER	14618
A	2112	ST. JOHN'S UNIVERSITY		GRAND CENTRAL UTOPIA PARKWAYS	JAMAICA	11432
X	2118	ST. JOSEPH'S HOSP HEALTH CTR		301 PROSPECT AVE.	SYRACUSE	13203
A	21169	ST. JOSEPH'S HOSPITAL		555 EAST MARKET STREET	ELMIRA	14902
A	21141	ST. LAWRENCE UNIVERSITY			CANTON	13617
A	2171	ST. LUKE'S HOSPITAL CENTER		AMSTERDAM AT W. 114TH ST.	NEW YORK	10025
X	2125	ST. MARY'S HOSPITAL		89 GENESEE ST.	ROCHESTER	14611
A	2164	ST. VINCENT'S HOSP & MED CNTR		153 W. 11TH ST.	NEW YORK	10011
A	2137	STANDARD BRANDS, INC.		625 MADISON AVE.	NEW YORK	10022
X	2114	STARK, DR. DM		ROCKEFELLER UNIV. 66TH ST & YO	NEW YORK	10021
A	21136	STATE UNIVERSITY OF NEW YORK		UNIVERSITY PLAZA RM. 415-5	ALBANY	12201
A	21168	STERILIZATION TECHNICAL SERVICES, INC.		3000 WINTON RD. S. BLDG. C	ROCHESTER	14623
A	2111	STERLING DRUG, INC.		COLUMBIA TURNPIKE	RENSSELAER	12144
A	21159	SURGICAL DESIGN CORP		24-05 JACKSON AVE	LONG ISLAND CITY	11101
A	21128	SYRACUSE UNIVERSITY		130 COLLEGE PL.	SYRACUSE	13210
A	21123	THE N.Y. SOCIETY FOR THE RELIEF OF THE RUPTURED & THE CR		541 E 71ST	NEW YORK	10021
X	218	THE NEW BLOOD CENTER		310 E. 67 ST.	NEW YORK	10021
A	217	TISSUE CULTURE ASSOC., INC.	W. ALTON JONES CELL SCIENCE CT., INC	7811 KNOX AVE. 3 RD	LAKE PLACID	12946
A	2170	TRUDEAU INSTITUTE, INC.		ALGONQUIN AVE.	SARANAC LAKE	12983
A	21104	TRUSTEES OF COLUMBIA UNIV.		BOX 20, LOW MEMORIAL LIBRARY	NEW YORK	10023
A	21109	UNIVERSITY OF ROCHESTER		RIVER BLVD.	ROCHESTER	27
A	213	USV PHARMACEUTICAL CORP		1 SCARSDALE RD.	TUCKAHOE	1
A	2134	WESTWOOD PHARMACEUTICALS, INC.		468 DEWITT ST.	BUFFALO	3
A	2188	WILSON MEM HOSP HEART-LUNG LB			JOHNSON CITY	90
A	21132	YESHIVA UNIVERSITY		55 FIFTH AVE.	NEW YORK	10003

NORTH CAROLINA

[illegible]	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	5520	AJINOMOTO U.S.A., INC.		4020 AJINOMOTO DRIVE	RALEIGH	27610
A	588	BURROUGHS WELLCOME CO.		3030 CORNWALLIS RD.	RES. TRIANGLE PARK	27709
A	5814	CARNIVORE EVOLUTIONARY RESEARCH INSTITUTE		ROUTE 3 BOX 180	PITTSBORO	27312
A	5819	CENTRAL CAROLINA TECH INST		1105 KELLY DR	SANFORD	27330
A	5817	CHEMICAL INDUSTRY INST. OF TOXICOLOGY (CIIT)		PO BOX 12137	RES. TRIANGLE PARK	27709
A	583	DUKE UNIVERSITY			DURHAM	27706
A	5813	GREER LABORATORIES, INC.		BOX 800	LENOIR	28645
A	5818	HEINEMAN MED. RES. CNTR		CHARLOTTE MEMORIAL HOSP	CHARLOTTE	28234
A	5811	LORILLARD RESEARCH CENTER		420 ENGLISH ST.	GREENSBORO	27420
A	586	N. CAROLINA STATE UNIVERSITY		P.O. BOX 5356	RALEIGH	27607
A	552	RESEARCH TRIANGLE INSTITUTE		PO BOX 12194	RES. TRIANGLE PARK	27709
A	5810	SCHOOL OF MED./EAST CAROLINA UNIV.		ANIMAL RESOURCES CENTER	GREENVILLE	27834
A	587	UNIV. OF N.C. AT CHARLOTTE			CHARLOTTE	28208
A	5812	UNIV. OF N.C. AT WILMINGTON		7205 WRIGHTSVILLE AVE.	WILMINGTON	28401
A	584	UNIVERSITY OF NORTH CAROLINA			CHAPEL HILL	27514
A	581	WAKE FOREST UNIVERSITY			WINSTON-SALEM	27109
A	5516	WESTERN CAROLINA UNIVERSITY			CULLOWHEE	28723

NORTH DAKOTA

[illegible]	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	453	MINOT STATE COLLEGE			MINOT	58701
A	452	NORTH DAKOTA STATE UNIVERSITY			FARGO	58102
A	451	UNIVERSITY OF NORTH DAKOTA		SCHOOL OF MEDICINE	GRAND FORKS	58201

OHIO

[illegible]	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	31R80	ADRIA LABORATORIES, INC.		4071 ST. RT. 161 E.	PLAIN CITY	43064
A	31R33	AKRON CITY HOSPITAL		525 E. MARKET ST.	AKRON	44309
A	3151	AKRON GENERAL MEDICAL CENTER		400 WABASH AVE.	AKRON	44307
A	31R72	ALLERGY LABS. OF OHIO, INC.		925 E. 11 AVE.	COLUMBUS	43211
A	31R74	BALDWIN-WALLACE COLLEGE			BEREA	44017
A	31R21	BATELLE MEMORIAL INSTITUTE		505 KING AVE.	COLUMBUS	43201
A	31R47	BEN VENUE LABS. INC		270 NORTHFIELD RD	BEDFORD	44146
A	31R28	BOWLING GREEN STATE UNIV.			BOWLING GREEN	43402
A	31R39	CAPITAL UNIVERSITY			COLUMBUS	43209
A	31R38	CASE WESTERN RESERVE UNIVERSITY		UNIVERSITY CIRCLE	CLEVELAND	44106
A	31R66	CENTRAL STATE UNIVERSITY		BANNEKER SCIENCE HALL	WILBERFORCE	45384
A	31R73	CHARLES F KETTERING RES. LAB.		150 E. SOUTH COLLEGE ST.	YELLOW SPRINGS	45387
A	31R11	CHILDREN'S HOSPITAL		ELLAND AVE. & BETHESDA	CINCINNATI	45229
A	31R13	CHILDREN'S HOSPITAL		561 S 17TH ST	COLUMBUS	43205
A	31R36	CHILDREN'S HOSPITAL OF AKRON		BUCHTEL AVE. AT BOWERY ST.	AKRON	44308
A	31R67	CHRIST HOSP INST. OF MED RES		2141 AUBURN AVE.	CINCINNATI	45219
A	31R69	CINCINNATI MILACRON, INC.		4701 MARBURG AVE.	CINCINNATI	45209
A	31R17	CLEVELAND CLINIC FOUNDATION		2020 E. 33RD ST.	CLEVELAND	44120
A	31R01	CLEVELAND RES. INST. AT ST. VINCENT'S CHARITY HOSP		2351 E. 22ND ST.	CLEVELAND	44115
A	31R58	CLEVELAND STATE UNIVERSITY		1983 E. 24TH ST.	CLEVELAND	44115
A	31R64	COLLEGE OF WOOSTER			WOOSTER	44691
A	31R88	COLUMBUS TECHNICAL INSTITUTE		550 E. SPRING ST.	COLUMBUS	43215
A	31R89	CREATIVE BIOLOGY LAB., INC.		3070 CLEVELAND-MASSILLON RD.	BARBERTON	44203
A	31R67	CUYAHOGA COMMUNITY COLLEGE		700 CARNEGIE AVE.	CLEVELAND	44115
A	31R42	DENISON UNIVERSITY			GRANVILLE	43023
A	31R81	DIAMOND SHAMROCK CORP		PO BOX 348	PAINESVILLE	44077
A	31R44	ELYRIA MEMORIAL HOSPITAL		630 E. RIVER ST.	ELYRIA	44035
A	31R03	FAIRVIEW GENERAL HOSPITAL		18101 LORAIN AVE.	CLEVELAND	44111
A	31R48	GIBCO DIVISION		P.O. BOX 200	CHAGRIN FALLS	44022
A	31R23	GOOD SAMARITAN HOSPITAL		3217 CLIFTON AVE.	CINCINNATI	45220
A	31R57	GRADY INVESTMENTS, INC.		3211 WINTON RD	CINCINNATI	45231
A	31R09	HILL TOP RESEARCH, INC.			MIAMIVILLE	45147
A	31R53	HIRAM COLLEGE BIOLOGICAL STA.		PO BOX 1938	HIRAM	44234
A	31R83	HURON ROAD HOSPITAL		13951 TERRACE ROAD	CLEVELAND	44112
A	31R50	JOHN CARROLL UNIVERSITY			CLEVELAND	44118
A	31R30	KENT STATE UNIVERSITY			KENT	44240
A	31R70	LAKELAND COMMUNITY COLLEGE			MENTOR	44060
A	31R83	MARIETTA COLLEGE			MARIETTA	45750
A	31R99	MAXSON, J A		HOCKING TECHNICAL COLLEGE	NELSONVILLE	45764
A	31R43	MEDICAL COLLEGE OF OHIO		PO BOX 6190	TOLEDO	43614
A	31R84	MERRELL DOW PHARMACEUTICALS INC.		110 E. AMITY RD.	CINCINNATI	45215
A	31R29	MIAMI UNIVERSITY		OFFICE OF THE PRESIDENT	OXFORD	45056
A	31R60	MIAMI VALLEY HOSPITAL		1 WYOMING ST.	DAYTON	45409
A	31R25	MT. SINAI HOSPITAL		UNIVERSITY CIRCLE	CLEVELAND	44106
A	31R78	MUSKINGUM COLLEGE			NEW CONCORD	43762
A	31R68	NORTH AM. SCIENCE ASSOC., INC		2261 TRACY RD.	NORTHWOOD	43605
A	31R82	NORTHEASTERN OHIO UNIV		RT 44	ROOTSTOWN	44272
A	31R35	OBERLIN COLLEGE			OBERLIN	44704
A	31R93	OHIO AGRICULTURAL RES. & DEVELOPMENT CNTR			WOOSTER	44691
A	31R85	OHIO COLLEGE OF PODIATRIC MEDICINE		10515 CARNEGIE AVE.	CLEVELAND	44106
A	31R86	OHIO DEPARTMENT OF HEALTH		1571 PERRY ST., PO BOX 2568	COLUMBUS	43216
A	31R40	OHIO NORTHERN UNIVERSITY			ADA	45810
A	31R14	OHIO STATE UNIVERSITY		190 N OVAL DR	COLUMBUS	43210
A	31R62	OHIO UNIVERSITY			ATHENS	45701
A	31R49	OHIO WESLEYAN UNIVERSITY			DELAWARE	43015
A	31R71	OTTERBEIN COLLEGE			WESTERVILLE	43081
A	31R46	RHONE-POULENC INC.		ROUTE 250	ASHLAND	44805
A	31R10	PROCTOR & GAMBLE CO.		PO BOX 39175	CINCINNATI	45239
A	31R84	SHADRACK BIOLOGICALS		7580 CHAGRIN RD	CHAGRIN	44022

OHIO—Continued

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP C
A	31R16	SPRINGBORN INST. FOR BIORESEARCH, INC.		563 N. BROADWAY ST.	SPENCERVILLE	[illegible]
A	31R31	ST. LUKE'S HOSPITAL		11311 SHAKER BLVD.	CLEVELAND	44104
A	31R62	ST. THOMAS HOSPITAL OF AKRON, (OHIO)		444 NORTH MAIN ST.	AKRON	44310
A	31R61	ST. VINCENT HOSPITAL & MEDICAL CENTER		2213 CHERRY ST.	TOLEDO	43608
A	31R19	UNIVERSITY OF AKRON			AKRON	44304
A	31R27	UNIVERSITY OF CINCINNATI		CLIFTON AVE.	CINCINNATI	45221
A	31R79	UNIVERSITY OF DAYTON		300 COLLEGE PARK	DAYTON	45469
A	31R06	UNIVERSITY OF TOLEDO		2801 W. BANCROFT ST.	TOLEDO	43606
A	31R89	WIL RESEARCH LABS., INC		3154 EXON AVE.	CINCINNATI	45241
A	31R81	WITTENBERG UNIVERSITY			SPRINGFIELD	45501
A	31R56	WRIGHT STATE UNIVERSITY		COL. GLENN HIGHWAY	45435	45435
A	31R65	XAVIER UNIVERSITY		VICTORY PARKWAY	CINCINNATI	45207
A	31R66	YOUNGSTOWN STATE UNIVERSITY		410 WICK AVE.	YOUNGSTOWN	44503

OKLAHOMA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	7312	DEAN MCGEE EYE INST.		908 STANTON YOUNG DR	OKLA CITY	73104
A	7313	IMMUNO-MYCOLOGICS, INC.		P.O. BOX 1151	NORMAN	73070
A	7314	NORTHEASTERN STATE UNIVERSITY		ANIMAL CARE FACILITY	TAHLEQUAH	74464
A	731	OK STATE UNIV/AGR. & APP SCI			STILLWATER	74074
X	734	OKLAHOMA CITY UNIVERSITY		2501 N. BLACKWELDER	OKLAHOMA CITY	73106
A	7310	OKLAHOMA COLLEGE OF OSTEOPATHIC MED. & SURGERY		1111 W. 17TH ST.	TULSA	74101
A	732	OKLAHOMA MEDICAL RESEARCH FOUNDATION		825 NE 13TH ST.	OKLAHOMA CITY	73104
A	738	OKLAHOMA STATE DEPT. OF HEALTH -		1000 N. E. 10TH	OKLAHOMA	73124
A	736	OKLAHOMA STATE DEPT. OF HEALTH -		1000 N. E. 10TH	OKLAHOMA	73124
A	7311	ORAL ROBERTS UNIVERSITY	BIOMEDICAL RESEACH CTR	7777 S LEWIS	TULSA	74171
A	733	SOUTHWESTERN OKLAHOMA ST. UNIV.		SCHOOL OF PHARMACY	WEATHERFORD	73096
A	737	UNIVERSITY OF OKLAHOMA		660 PARRINGTON OVAL	NORMAN	73069

OREGON

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	3221	AMERICAN COLLEGE OF SURGEONS		577 EAST 12TH ST. NO. 450	EUGENE	97451
A	3219	ANIMAL FOUNDATION INC		921 RIVER AVENUE	EUGENE	97404
A	3218	ANIMAL HEALTH LABORATORY		536 CAPITOL ST NE	SALEM	[illegible]
A	3215	BIOPRODUCTS, INC.		BOX 429	WARRENTON	[illegible]
A	322	GOOD SAMARITAN HOSP & MED CTR		1015 NW 22ND AVE.	PORTLAND	[illegible]
A	3220	LINFIELD COLLEGE			MCMINNVILLE	[illegible]
A	3210	OR REGIONAL PRIMATE RES. CTR.		505 NW 185TH AVE.	BEAVERTON	97006
A	325	OREGON STATE UNIVERSITY			CORVALLIS	97331
A	323	PACIFIC UNIVERSITY			FOREST GROVE	97116
A	3213	PCL/RIA, INC.		10700 N.E. SANDY BLVD.	PORTANO	97220
A	327	PORTLAND STATE UNIVERSITY		PO BOX 751	PORTLAND	97207
A	3212	PROVIDENCE MEDICAL CENTER		700 N.E. 47TH AVE.	PORTLAND	97213
A	3216	REED COLLEGE		3203 S.E. WOODSTOCK BLVD	PORTLAND	97202
A	326	UNIV. OF OREGON DENTAL SCHOOL		611 SW CAMPUS DR.	PORTLAND	97201
A	321	UNIV/OREGON MED SCHOOL		3181 SW SAM JACKSON PK RD	PORTLAND	97201
A	328	UNIVERSITY OF OREGON			EUGENE	97403
X	3214	WESTERN OREGON STATE COLLEGE		346 MONMOUTH AVE.	MONMOUTH	97361
A	3217	WILLAMETTE LABORATORIES		2480 NE FREMONT	PORTLAND	97212

PENNSYLVANIA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	23110	ACADEMY OF NATURAL SCIENCES		19TH AND THE PARKWAY	PHILADELPHIA	19103
A	2332	ALBERT EINSTEIN MEDICAL CNTR.		YORK & TABOR RDS.	PHILADELPHIA	19141
A	2322	ALLEGHENY-SINGER RESEARCH CORP		320 E. NORTH AVE.	PITTSBURGH	15212
A	2385	ALLEN PRODUCTS CO., INC.		PO BOX 2187 RD #3	ALLENTOWN	18001
A	23102	ALLENTOWN AND SACRED HEART HOSPT. CT.		1200 SOUTH CEDAR CREST BLVD	ALLENTOWN	18105
A	2398	ARGUS RESEARCH LABORATORIES, INC.		2025 RIDGE RD	PERKASIE	18944
A	23106	BIOLOGICAL CORPORATION OF AMERICA		1230 WILSON DRIVE	WEST CHESTER	19380
A	2382	BIOSEARCH, INC.		PO BOX 8588	PHILDELPHIA	19101
A	2346	BUCKNELL UNIVERSITY			LEWISBURG	17837
A	2379	BUCKSHIRE CORPORATION		2025 RIDGE RD.	PERKASIE	18944
A	2352	CALIFORNIA STATE COLLEGE			CALIFORNIA	15419
A	2342	CANNON LABORATORIES		PO BOX 3627	READING	19605
A	2324	CARNEGIE-MELLON UNIVERSITY		5000 FORBES AVE	PITTSBURGH	15213
A	2353	CENTRE SQUARE VETERINARY CLINIC		1030 DEKALB PIKE	CENTRE SQUARE	19422
A	2333	CHILDREN'S HOSP OF PHILA.		1 CHILDRENS CENTER	PHILAGELPHIA	19104
A	2381	CHILDREN'S HOSP. OF PITT.		125 DESOTO ST	PITTSBURGH	15213
A	2363	COLLEGE MISERICORDIA			DALLAS	18612
A	2384	CONNAUGHT LABORATORIES, INC.		RT. 611	SWIFTWATER	18370
A	2329	CONTRIBUTORS TO THE PA HOSP.		8TH & SPRUCE STS.	PHILADELPHIA	19107
A	2370	CORATOMIC, INC.		PO BOX 434	INDIANA	15701
A	2341	DALMATIAN RESEARCH FOUND.		720 WOODBURY RD	YORK	17403
A	2394	DEBORAH HOSPITAL FOUNDATION	DEBORAH HEART & LUNG CTR.	1715 ARCH STREET	PHILADELPHIA	19103
X	2314	DONALD GUTHRIE FND. FOR MEDICAL RES., INC.		200 S. WILBUR AVE.	SAYRE	18840
A	2326	DREXEL UNIVERSITY		32ND & CHESTNUT ST.	PHILADELPHIA	[illegible]
A	2313	DUQUESNE UNIVERSITY			PITTSBURGH	[illegible]
A	23108	E. I. DUPONT DE NEMOURS & CO., INC.		GLENOLDEN LABORATORY	GLENOLDEN	[illegible]

PENNSYLVANIA—Continued

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
X	2310	EASTERN PA PSYCHIATRIC INST.		HENRY AVE. & ABBOTTSFORD	PHILADELPHIA	19129
A	2396	EYE AND EAR HOSPITAL		230 LOTHROP STREET	PITTSBURGH	15213
A	2367	FEDERATED MEDICAL RESOURCES		RD 2	HONEY BROOK	19344
A	2387	FISHER SCIENTIFIC CO.		711 FORBES AVE.	PITTSBURGH	15219
A	2377	GENERAL ELECTRIC CO.		VALLEY FORGE SPACE CENTER	KING OF PRUSSIA	19406
A	23109	GULF RESEARCH & DEVELOPMENT COMPANY	GULF LIFE SCIENCES CENTER	P.O. BOX 3240	PITTSBURGH	15230
A	2331	HAHNEMANN MED. COLLEGE & HOSP		230 N. BROAD ST.	PHILADELPHIA	19102
A	23104	HALLORAN, W. ROBERT & ELIZABETH		RT. 29, PO. BOX 111	SOUTH MONTROSE	18843
A	2378	HARCUM JUNIOR COLLEGE		MORRIS & MONTGOMERY AVE.	BRYN MAWR	19010
A	2376	INDIANA UNIV. OF PENNSYLVANIA			INDIANA	15701
A	2358	INSTITUTE FOR CANCER RESEARCH		7701 BURHOLME AVE.	PHILADELPHIA	19111
A	236	INSTITUTE, GEISINGER MEDICAL CNTR.		THE GEISINGER MEDICAL CNTR.	DANVILLE	17821
A	233	LANKENAU HOSPITAL		LANCASTER & CITY LINE AVES.	PHILADELPHIA	19151
A	2364	LEHIGH UNIVERSITY			BETHLEHEM	18015
X	2371	LINCOLN UNIVERSITY			LINCOLN UNIVERSITY	19352
A	23105	LORD CORPORATION		2101 PENINSULA DRIVE	ERIE	16512
A	2350	LYCOMING COLLEGE			WILLIAMSPORT	17701
A	2389	M.B. RESEARCH LAB INC.		PO BOX 178 STEINBERG WENTZ RD	SPINNERSTOWN	18968
A	2381	M.B. RESEARCH LABS., INC.		STEINSBURG & WENTZ ROADS	SPINNERSTOWN	18968
A	2390	MAGEE-WOMENS HOSPITAL		FORBES & HALKET STS.	PITTSBURGH	15213
A	2315	MCNEIL LABORATORIES, INC.		CAMP HILL RD.	FT. WASHINGTON	19034
A	2375	MEDIAN SCH. OF ALLIED HEALTH CAREERS		121 9TH ST.	PITTSBURGH	15222
A	230	MEDICAL COLLEGE OF PENNA.		3300 HENRY AVE.	PHILADELPHIA	19129
A	2338	MERCY CATHOLIC MEDICAL CENTER		LANSDOWNE AVE.	DARBY	19023
A	2386	MONELL CHEMICAL SENSES CTR		3500 MARKET ST	PHILADELPHIA	19104
A	2392	MONTEFIORE HOSPT. ASSOC.WSTN. PA		3459 FIFTH AVE	PITTSBURGH	15213
A	2357	MORAVIAN COLLEGE		MAIN & ELIZABETH AVE.	BETHLEHEM	18018
A	2369	PA COLLEGE OF PODIATRIC MED.		8TH AT RACE ST.	PHILADELPHIA	19107
A	23100	PENN COLLEGE OF OPTOMETRY		1200 WEST GODFREY AVE	PHILADELPHIA	19141
A	2384	PENNSYLVANIA DEPT. OF HEALTH		PICKERING WAY & WELSH POOL RD.	LIONVILLE	19353
A	2321	PENNSYLVANIA STATE UNIVERSITY		207 OLD MAIN	UNIVERSITY PARK	16802
A	2335	PENNWALT CORPORATION		3 PENN CENTER	PHILADELPHIA	19102
A	2354	PENROSE RESEARCH LABORATORY		34TH ST. & GIRARD AVE.	PHILADELPHIA	19104
A	2380	PHARMACHEM CORP.		BOX 1036 719 STEFKO BLVD.	BETHLEHEM	18018
A	2345	PHARMAKON LABORATORIES		PO. BOX 313	WAVERLY	18471
A	23107	PHARMAKON RESEARCH INTERNATION, INC.			WAVERLY	18471
A	2325	PHILA. COLL. OF OSTEOP. MED.		4150 CITY LINE AVE.	PHILADELPHIA	19131
A	231	PHILADELPHIA COL. OF PHARMACY & SCIENCE		43RD ST. & KINGSESSING AVE.	PHILADELPHIA	19104
A	2319	PRESBYTERIAN-UNIVERSITY OF PA		51 N. 39TH ST.	PHILADELPHIA	19104
A	2397	PRODUCT INVESTIGATIONS, INC.	HALLOWELL LABORATORIES	151 EAST TENTH AVE	CONSHOHOCKEN	19428
A	2337	ROHM & HAAS COMPANY		NORRISTOWN & MCKEAN RDS.	SPRINGHOUSE	19477
A	2395	SACKS, L.	SACKS FARM	RD3	EVANS	16033
A	2312	SMITHKLINE CORPORATION		1500 SPRING GARDEN ST.	PHILADELPHIA	19101
A	2368	ST. VINCENT COLLEGE			LATROBE	15650
A	2348	SUSQUEHANNA UNIVERSITY			SELINSGROVE	17870
X	2362	SYNDOT LABORATORIES		1075 NEW DEHAVEN RD.	W. CONSHOHOCKEN	19428
A	2327	TEMPLE UNIVERSITY		BROAD & MONTGOMERY STS.	PHILADELPHIA	19122
A	2385	THE GRADUATE HOSPITAL		18TH & LOMBARD STS.	PHILADELPHIA	19146
A	238	THE MERCY HOSPITAL OF PITTSBURGH		1400 LOCUST ST.	PITTSBURGH	15219
A	2311	THOMAS JEFFERSON UNIVERSITY		1025 WALNUT ST.	PHILADELPHIA	19107
A	2318	UNIV. HEALTH CENTER OF PITTSBURGH		TERRACE & DESOTO STS.	PITTSBURGH	15213
A	2323	UNIVERSITY OF PENNSYLVANIA		101 COLLEGE HALL	PHILADELPHIA	19104
A	23103	VILLANOVA UNIVERSITY		MENDEL HALL ROOM G-11	VILLANOVA	19085
A	2349	W. L. CAPPEL LABS., INC.		THUD RIDGE FARM	COCHRANVILLE	19330
A	2328	WESTINGHOUSE CORP.		1310 BEULAH RD.	PITTSBUGH	15235
A	23101	WHITE EAGLE LABORATORIES, INC.		2003 LOWER STATE RD	DOYLESTOWN	18901
A	239	WILLIAM H. RORER, INC.		500 VIRGINIA DR.	FORT WASHINGTON	19034
A	2366	WISTAR INSTITUTE		36TH & SPRUCE STS.	PHILADELPHIA	19104
A	2316	WYETH LABORATORIES, INC.		PO BOX 8299	PHILADELPHIA	19101

PUERTO RICO

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	94R11	ANA V. FERRAN			PONCE	00731
A	94R8	DADE DIAGNOSTICS INC		RD 11.5 KM 22.6	AGUADA	00602
A	94R2	ELI-LILLY INDUSTRIES, INC.		PO BOX 71325	SAN JUAN	00936
A	94R4	EX-LAX, INC.		CALL BOX 1020	HUMACAO	00661
X	94R3	INSTITUTE OF HEALTH LABS.		PO BOX 10427, CAPARRA HEIGHTS	SAN JUAN	00922
A	94R5	OMNI RESEARCH, INC.		PO BOX 1156	SAN GERMAN	00753
A	94R10	PONCE SCHOOL OF MEDICINE		AVENIDA UNIVERSIDAD	PONCE	00731
A	94R9	UNIVERSIDAD CENTRAL DEL CARIBE		SCHOOL OF MEDICINE, BOX 935	CAYEY	00633
A	94R1	UNIVERSITY OF PUERTO RICO			RIO PIEDRAS	00931

RHODE ISLAND

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	153	BROWN UNIVERSITY		79 WATERMAN ST. BROWN STA.	PROVIDENCE	02912
A	1511	CURRAN, ALTON J. M.D.		314 ANGELL ST.	PROVIDENCE	02906
A	158	MEMORIAL HOSPITAL		PROSPECT ST.	PAWTUCKET	02860
A	155	MIRIAM HOSPITAL		164 SUMMIT AVENUE	PROVIDENCE	02906
A	1513	PROVIDENCE COLLEGE			PROVIDENCE	02918
A	1510	RHODE ISLAND COLLEGE			PROVIDENCE	02908
A	152	RHODE ISLAND HOSPITAL		593 EDDY ST.	PROVIDENCE	02903
A	157	ROGER WILLIAMS GENERAL HOSP.		825 CHALKSTONE AVE.	PROVIDENCE	02908
A	154	UNIVERSITY OF RHODE ISLAND			KINGSTON	02881

SOUTH CAROLINA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	562	CLEMSON UNIVERSITY			CLEMSON	29631
A	561	MEDICAL COLLEGE OF S. CAROLINA		171 ASHLEY AVE.	CHARLESTON	29403
A	566	SAVANNAH RIVER LABORATORY			AIKEN	29808
A	564	TRI-COUNTY TECHNICAL COLLEGE		BOX 87	PENDLETON	29670
A	563	UNIVERSITY OF SOUTH CAROLINA			COLUMBIA	29208
A	565	WINTHROP COLLEGE			ROCK HILL	29733

SOUTH DAKOTA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	462	SOUTH DAKOTA STATE UNIVERSITY			BROOKINGS	57006
A	461	UNIVERSITY OF SOUTH DAKOTA			VERMILLION	57069

TENNESSEE

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	538	BAPTIST MEMORIAL HOSPITAL		899 MADISON AVE.	MEMPHIS	38103
A	531	BEECHAM LABORATORIES		501 FIFTH ST.	BRISTOL	37620
A	6311	EAST TENNESSEE STATE UNIV			JOHNSON CITY	37601
A	532	MEHARRY MEDICAL COLLEGE		1005 18TH AVE. NORTH	NASHVILLE	37208
A	536	MEMPHIS STATE UNIVERSITY			MEMPHIS	38111
A	533	OAK RIDGE ASSOC. UNIVERSITIES		PO BOX 117	OAK RIDGE	37830
A	6310	PLOUGH, INC.		3030 JACKSON AVE.	MEMPHIS	38112
A	537	ST. JUDE CHILDREN'S RES HOSP		PO BOX 318	MEMPHIS	38101
A	534	UNIVERSITY OF TENNESSEE			KNOXVILLE	37916
A	535	VANDERBILT UNIVERSITY			NASHVILLE	37203

TEXAS

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	7466	ACORN RESEARCH LAB.		3410 OWEN	AUSTIN	78705
A	7411	ALCON LABORATORIES, INC.		PO BOX 1959	FORT WORTH	76101
A	7462	AM BIOTECHNIQUES, INC.		212 EAST CENTRAL	BELTON	76513
A	7442	AMF/BIOLOGICAL & DIAGNOSTIC PRODUCTS CO.		1015 N. AUSTIN	SEGUIN	78155
A	7439	BAYLOR COLLEGE OF DENTISTRY		800 HALL ST.	DALLAS	75235
A	7429	BAYLOR UNIVERSITY			WACO	76703
A	7418	BAYLOR UNIVERSITY COLLEGE OF MEDICINE		1200 MOURSUND AVE.	HOUSTON	[illegible]
A	7434	BROWNSVILLE ANIMAL RES. CNTR.		1810 CENTRAL AVE.	BROWNSVILLE	[illegible]
A	7430	CARDIOVASULAR RESEARCH LAB.		PO. BOX 225999	DALLAS	[illegible]
A	7444	CARWILE, DVM, HF	BETHYL LABS INC.	PO. BOX 821	MONTGOMERY	77356
A	7454	DALLAS CO. COMM. COLLEGE DIST		701 ELM	DALLAS	75202
A	7443	FRANKLIN LABORATORIES		PO BOX 989	AMARILLO	79105
A	7463	GAMMA BIOLOGICALS, INC.		3700 MANGUM RD.	HOUSTON	77092
A	7438	HELENA LABORATORIES		1530 LINDBERGH DR.	BEAUMONT	77704
A	7461	IMMUNO-MODULATORS LABORATORIES LTD.		PO. BOX 1279	STAFFORD	77477
A	7467	INTERMEDICS, INC.		PO BOX 617	FREEPORT	77541
A	7453	JOHN H. SHEETS, M.D., ASSOC.		PO BOX 7016	ODESSA	79760
A	7469	KALLESTAD	KALLESTAD LABORATORIES, INC.	2000 AUSTIN NAT'L BANK TOWER	AUSTIN	78701
A	7445	LAMAR UNIVERSITY		BOX 10037 BIOLOGY DEPT	BEAUMONT	77710
A	7433	NORTH TEXAS STATE UNIVERSITY		BOX 5218 NT STATION	DENTON	76203
A	7452	PAN AMERICAN UNIVERSITY			EDINBURG	78539
A	7431	RICE UNIVERSITY		PO BOX 1892	HOUSTON	77001
A	7408	SCOTT & WHITE MEMORIAL HOSP		2401 S. 31ST ST.	TEMPLE	76501
A	7432	SOUTHERN METHODIST UNIVERSITY			DALLAS	75222
A	7403	SOUTHWEST FOUNDATION FOR RES. & ED.		PO. BOX 28147	SAN ANTONIO	78284
A	7426	SOUTHWEST RESEARCH INSTITUTE		3500 CULEBRA RD.	SAN ANTONIO	78206
A	7448	SOUTHWEST TEXAS STATE UNIV.			SAN MARCOS	78666
A	7480	ST JOSEPH HOSPITAL	SURGICAL TRAINING LAB.	1800 CALHOUN	HOUSTON	77002
A	7406	ST. JOSEPH'S HOSPITAL		1919 LA BRANCH	HOUSTON	77002
A	7415	ST. PAUL HOSPITAL		5909 HARRY HINES	DALLAS	75235
A	7449	STILLMEADOW, INC.		3223 LACOSTA DR.	MISSOURI CITY	77459
A	7407	TECHNOLOGY, INC.		511 WEST RAPSODY	ST. ANTONIO	78215
A	7412	TEXAS A&M UNIVERSITY		LARR FACILITY	COLLEGE STATION	77843
A	7446	TEXAS CHRISTIAN UNIVERSITY			FT. WORTH	76129
A	7437	TEXAS HEART INSTITUTE			HOUSTON	77002
A	7401	TEXAS RESEARCH INSTITUTE OF MENTAL SCIENCES		1300 MOURSUND	HOUSTON	77025
A	7445	TEXAS STATE TECHNICAL INST.		JAMES CONNALLY CAMPUS	WACO	76705
A	7447	TEXAS TECH UNIVERSITY			LUBBOCK	79409
A	7440	TEXAS WOMAN'S UNIVERSITY		BOX 22871, TWU STATION	DENTON	76204
A	7427	TRINITY UNIVERSITY		715 STADIUM DR.	SAN ANTONIO	78212
A	7450	TX TECH. UNIV HEALTH SCI CNTR		PO BOX 4569	LUBBOCK	79409
A	7420	UNIVERSITY OF HOUSTON		3801 CULLEN BLVD.	HOUSTON	77004
A	7428	UNIVERSITY OF TEXAS SYSTEM		PO BOX 7999	AUSTIN	78712
A	7458	VACCINES, INC.		2114 SO. 1ST	TEMPLE	76501
A	7438	WADLEY INSTS OF MOLECULAR MED		3000 HARRY HINES	DALLAS	75235
A	7464	ZANETTI, P H M.D.		1224 THIRD ST.	CORPUS CHRISTI	78404
A	7436	ZOECON INDUSTRIES, INC.		12200 DENTON DR.	DALLAS	75234

UTAH

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP
A	573	BRIGHAM YOUNG UNIVERSITY		RM 679 WIDTSOE BLDG	PROVO	[illegible]
A	575	INTERMOUNTAIN LABORATORIES		670 E. 7200 SOUTH	MIDVALE	84047
A	574	LATTER-DAY SAINTS HOSPITAL		325-8TH AVE.	SALT LAKE CITY	84103
A	579	MOECO		391 CHIPETA WAY SUITE B	SALT LAKE CITY	84108

UTAH—Continued

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	879	PRIMARY CHILDREN'S HOSPITAL		320 TWELFTH AVE.	SALT LAKE CITY	84103
A	871	UNIVERSITY OF UTAH		309 PARK BLDG.	SALT LAKE CITY	84112
A	8710	UNIVERSITY OF UTAH RES. INSTITUTE		520 WAKARA WAY	SALT LAKE CITY	84108
A	872	UTAH STATE UNIVERSITY			LOGAN	84321
A	878	WEBER STATE COLLEGE		3750 HARRISON BLVD.	OGDEN	84403

VERMONT

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	135	INST FOR MED RES/BENNINGTON		DEWEY ST.	BENNINGTON	05201
A	133	MIDDLEBURY COLLEGE			MIDDLEBURY	05753
A	131	UNIV OF VT & STATE AGRI. COLL			BURLINGTON	05401
A	139	VERMONT TECHNICAL COLLEGE		RANDOLPH CTR.	VERMONT	05061

VIRGINIA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	527	A.H. ROBINS CO. INC.		1211 SHERWOOD AVE.	RICHMOND	23220
A	5217	BLUE RIDGE COMMUNITY COLLEGE		PO BOX 80	WEYERS CAVE	24486
A	5214	COLLEGE OF WILLIAM AND MARY			WILLIAMSBURG	23185
A	529	EASTERN VA MEDICAL AUTHORITY		P.O. BOX 1980 OLNEY RD.	NORFOLK	23501
A	VAR20	ENVIRONMENTAL CONSULTANTS INC		1022 W 24TH ST	NORFOLK	23517
A	VAR21	FLOW LABORATORIES, INC		7655 OLD SPRINGHOUSE ROAD	MCLEAN	22102
A	5219	GEORGE MASON UNIVERSITY		4400 UNIVERSITY DR.	FAIRFAX	22030
A	5218	GLENN H. SHEPARD, M.D. INC.		314 MAIN ST.	NEWPORT NEWS	23601
A	VAR6	HAZELTON LAB., INC.		9200 LEESBURG TURNPIKE	VIENNA	22180
A	VAR24	HAZLETON RESEARCH PRIMATES		1946 ISAAC NEWTON SQUARE, WEST	RESTON	22090
A	52R22	HAZLETON/PRIMELABS, INC.		MONMOUTH COUNTY AIRPORT	FARMINGDALE	07727
X	5216	LYNCHBURG COLLEGE			LYNCHBURG	24504
A	5210	MELOY LABS., INC		6715 ELECTRONIC DR.	SPRINGFIELD	22151
A	5213	OLD DOMINION UNIVERSITY		PO BOX 6173	NORFOLK	23508
A	VAR23	UNIVERSITY OF RICHMOND		DEPT. OF BIOLOGY	UNIVERSITY OF RICHMOND	23173
A	VAR11	UNIVERSITY OF VIRGINIA	VIVARIUM FARM	MEDICAL CNTR/PO. BOX 450	CHARLOTTESVILLE	22908
A	VAR7	VA COMMONWEALTH UNIV.		12TH & BROAD STS.	RICHMOND	23219
A	525	VIRGINIA POLYTECHNIC INST.			BLACKSBURG	24061
A	5211	WASHINGTON AND LEE UNIVERSITY			LEXINGTON	24450

WASHINGTON

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	9120	BIO MEDICAL RESEARCH LABS.		1115 E. PIKE ST.	SEATTLE	98122
A	9123	CENTRAL WASHINGTON UNIVERSITY			ELLENSBURG	98926
A	919	EASTERN WASHINGTON STATE COLLEGE			CHENEY	99004
A	9118	FORT STEILACOOM COMMUNITY COLLEGE		BOX 99186	TACOMA	98499
A	9111	HOLLISTER-STIER LABORATORIES		BOX 3145 TERMINAL ANNEX	SPOKANE	99220
A	9122	LOWER COLUMBIA COLLEGE		1600 MAPLE ST	LONGVIEW	98632
A	916	PACIFIC NORTHWEST LABS.		PO BOX 999	RICHLAND	99352
A	915	PACIFIC NW RESEARCH FNDN.		1102 COLUMBIA ST.	SEATTLE	98104
A	9121	PHYSIO-CONTROL CORPORATION	EVERGREEN VET HOSPITAL	11811 WILLOWS RD	REDMOND	98052
A	913	PROVIDENCE HOSPITAL RES. CTR		528 18TH AVE.	SEATTLE	98158
A	9119	PUGET SOUND BLOOD CENTER		TERRY AT MADISON	SEATTLE	98104
A	9114	SEATTLE-KING CO. HEALTH DEPT.		1300 PUBLIC SAFETY BLDG.	SEATTLE	98104
A	9112	ST. JOSEPH HOSPITAL			TACOMA	98405
A	911	UNIVERSITY OF WASHINGTON			SEATTLE	98101
A	917	VIRGINIA MASON RESEARCH CNTR.		1000 SENECA ST.	SEATTLE	98101
A	912	WASHINGTON STATE UNIVERSITY			PULLMAN	99163
A	9117	WESTERN WASH. UNIV.			BELLINGHAM	98225
A	9110	WEYERHAEUSER COMPANY		PO BOX 420	CENTRALIA	98531

WEST VIRGINIA

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	54R3	FAIRMONT STATE COLLEGE		103 HUNT HAUGHT HALL	FAIRMONT	26554
A	54R4	MARSHALL UNIVERSITY			HUNTINGTON	25701
A	541	WEST VIRGINIA UNIVERSITY		MEDICAL CENTER	MORGANTOWN	26506
A	542	WV SCHOOL OF OSTEOP. MEDICINE		400 N. LEE ST.	LEWISBURG	24901

WISCONSIN

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	3515	ALTECH LABORATORIES		RT. 4 SYENE RD.	MADISON	53711
A	3523	APPLETON MEMORIAL HOSP. ASSOC		1818 N. MEADE ST.	APPLETON	54911
A	3519	BELLIN MEMORIAL HOSPITAL		744 S. WEBSTER AVE.	GREEN BAY	54301
A	3521	BELOIT COLLEGE			BELOIT	53511
X	354	CENT. WI CT FOR DEVEL. DISABLED		317 KNUTSON DR.	MADISON	53704
A	3512	FROMM LABORATORIES, INC.			GRAFTON	53024
A	3530	HAZLETON RALTECH, INC.		3301 KINSMAN BOULEVARD	MADISON	53704
A	3511	MARQUETTE UNIVERSITY		1217 W. WISCONSIN AVE.	MILWAUKEE	53233
A	358	MARSHFIELD MEDICAL FOUND.		630 S. CENTRAL AVE.	MARSHFIELD	54449
A	3529	MEDICAL COLLEGE OF WISCONSIN, INC.		8701 WATERTOWN PLANK RD	MILWAUKEE	53226
A	3528	MILWAUKEE CHILDREN'S HOSPITAL		1700 W. WISCONSIN AVE.	MILWAUKEE	53233
A	359	MT. SINAI HOSPITAL		948 N. 12TH ST.	MILWAUKEE	53233

WISCONSIN—Continued

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	3527	S.C. JOHNSON & SON, INC.		1525 HOWE ST.	RACINE	53403
A	3524	ST. JOSEPH'S HOSPITAL-LAB.		5000 W. CHAMBERS ST.	MILWAUKEE	53210
A	3516	VOC., TECH., & ADULT EDUC.		211 N. CARROLL ST.	MADISON	53703
A	3520	WISCONSIN DEPT. OF AGRICULTURE		801 W. BADGER RD.	MADISON	53713

WYOMING

STATUS	REGISTRATION NUMBER	NAME	DOING BUSINESS AS	ADDRESS	CITY	ZIP CODE
A	532	CASPER COLLEGE		125 COLLEGE DR.	CASPER	82601
A	534	EASTERN WYOMING COLLEGE			TORRINGTON	82240
A	533	LARAMIE CO. COMMUNITY COLLEGE		1400 EAST COLLEGE DR.	CHEYENNE	82001
A	531	UNIVERSITY OF WYOMING			LARAMIE	82070

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ETHICAL CONSIDERATIONS :

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TABLE OF CONTENTS

Introduction 1

I. The Religious and Moral Traditions 3

  A. God, Man, and Beast 3

  B. The Theologians on Animals 7

  C. Human and Animal Nature 11

II. The Moral Questions 15

  A. Moral Standing 16

  B. Moral Constraints 24

III. Moral Basis for Federal Legislation Protecting Animals 34

  A. Legislation Protecting Animals as Individuals 35

  B. Legislation Protecting Animal Species 48

IV. Animal Experimentation for Public Health and Safety 52

V. Summary and Conclusions 61

Appendix 65

Notes 70

Introduction

The range of opinion on the rights and wrongs of using animals to satisfy human needs is as broad as the political spectrum itself. At one far end, animals are thought to be dispossessed claimants to at least a portion of the stature and dignity now invested in men when we speak of human rights. It is only a matter of time, in the minds of some, before animals follow blacks and women as admitted applicants to a community which gives each being its proper respect. At the other extreme, human beings are taken to enjoy an authority over the lives and interests of animals as sweeping in scope and absolute in right as any that a master or autocrat ever claimed. From this perspective, expediency alone, not morality, dictates what we may do with animals.

To measure the distance between these extremes, one might begin with a recent brief for animal rights.

> If being alive is the basis for being a moral object, and if all other interests and needs are predicated upon life, then the most basic, morally relevant aspect of a creature is its life. We may correlatively suggest that any animal, therefore, has a right to life.[1]

Three scientists whose work with animals drew them into a televised exchange with Harvard philosopher Robert Nozick would seem to travel in an alien world.

> Nozick asked the scientists whether the fact that an experiment will kill hundreds of animals is ever regarded, by scientists, as a reason for not performing it. One of the scientists answered: "Not that I know of." Nozick pressed the question: "Don't the animals count at all?" Dr. A. Perachio, of the Yerkes Center, replied: "Why should they?", while Dr. D. Baltimore, of the Massachusetts Institute of Technology, added that he did not think that experimenting on animals raised a moral issue at all.[2]

Had Nozick pressed a third question, he might have found some common ground.

Of two experimental designs, both promising the same results with the same reliability, wouldn't one which did not require killing animals be preferable to one which did? Perhaps everyone would agree that it was; yet if economic expense rather than moral offense were the basis for some responses, the consensus secured would be more apparent than real. The two warring opinions might have reached an uneasy truce, but one which would hold together only as long as economic and moral considerations managed to coincide.

In morals as in politics, fortunately, the sense that most men have in common tends to shun extremes. Proposals born in legislatures and universities have more often than not mirrored this trend. The common sense view, in ethics and elsewhere, is at once the most defensible and the most difficult to defend. Pitted against extreme or esoteric positions, it has the advantage of broad acceptance, and the numbers on its side create a presumption in its favor. All disciplines begin with common sense, as the position most likely to be correct (if only in outline); they will end there, too, unless good reason can be given to abandon what most of us believe to be true.

Yet a presumption given only by the weight of opinion will not amount to a justification. A belief is not shown to be true simply by counting the votes of those who accept it. Some basis for received opinion, independent of the fact that it is received, must be found, and the difficulty lies here. Common sense is a centripetal intellectual force. It gravitates toward a characteristically complex middle ground which borrows its elements, with amendments, from the simpler extremes. This course is hazardous in two respects. First, it runs the risk of inconsistency. Propositions located at polar extremes will usually contradict one another, and a position which seeks to incorporate both may find itself embracing a contradiction. In the case of toxicity testing, for example, it may not at first seem possible to respect the interests of experimental animals and yet use them as tools for enhancing human health and safety.

The second risk is that consistency will be secured at a price too high, by way of a theoretically unattractive ad hoc device. It will always be possible in principle to reconcile two contradictory propositions by the simple expedient of making one an exception to the other. Thus we could just stipulate that the general rule against harming animals does not hold where they are used to test for toxicity. But it is one thing to say this and another to give a reason for it. Complex rules introduced for no reason other than to remove a particular inconsistency add clutter to one's point of view without shedding any light on the hard moral cases which it must address.

The working hypothesis for what follows will be that there is an identifiable common sense position on the morally appropriate use of animals in research, testing, and education. But that position must be carefully constructed, from traditional and contemporary resources, to preserve consistency and to locate support in principles that are not simply contrived to handle the special situation of animals. The common sense view, it turns out, is also the Congressional view.

I. The Religious and Philosophical Traditions

A) God, Man, and Beast

Interest in the moral status of animals is by no means peculiarly modern. The ancient religions had much to say about the place that animals were to occupy in the cosmic scheme of things. Oriental creeds were as a rule reluctant to draw a sharp distinction between the human and other species. All animal lives were judged worthy of protection. Some were thought to be sacred. The doctrine of transmigration[a] left still more room for caution: Any animal body might house a soul entitled to special care. For such reasons the prescribed dietary regimen in the East was frequently vegetarian. Modern influences have relaxed without wholly removing the grip that these beliefs have had on Oriental practice.

In the West a different tradition has taken root, one which seems to have assigned value to animals only as they serve human purposes. Judaeo-Christian doctrine appears to have condoned an indifferent if not openly exploitative attitude toward the lower animals.[3] The Genesis account, leaving the creation of man to the fifth day of God's labors, suggests that man is the culmination of that process, the last and most perfected of God's creatures. Man alone, of all living things, bears the likeness of God, and he receives the divine commission to exercise "dominion over the fish of the sea, and over the birds of the air, and over the cattle, and over all the earth, and over every creeping thing that creeps upon the earth."[4] Later, after the flood, God rewards Noah and his sons with this blessing: "Every moving thing that lives shall be food for you; and as I gave you the green plants, I give you everything."[5] A brief Talmudic story indicates that Judaic practice was to the same effect:

> A calf was being taken to the slaughterer, when it broke away, hid his head under Rabbi's skirts, and lowed in terror. "Go," said he, "for this wast thou created."[6]

A strict construction of these passages, however, would not warrant the inference that man is permitted to treat animals however he may please. Even when he inflicts suffering as a means to some human end, he is subject to the condition of use itself. Wanton cruelty would not be allowed. Nor will it be clear just what man's dominion includes until the terms of a model ruler-subject relation are spelled out. Man must presumably rule well, and the good ruler does not operate with a license to do whatever he wishes to those under his authority. Much depends, too, on whether human sovereignty over nature is to be thought absolute or limited in turn by the divine will. That will may have set some value on animals in addition to their utility for man.

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[a] The various forms of this doctrine share the thesis that a single, continuous immaterial individual may pass from one body to another, which may be of the same or a different species. If the latter, its conduct in the earlier incarnation may determine the kind of body it inhabits next.

In a number of passages the scriptures seem to place a rein on our employment of animals. Genesis confirms that God had already judged his creation good - that is, possessed of some value - before he created man.[7] Note also the coda to the blessing given Noah: "...as I gave you the green plants." Fruits and vegetables, but not animals, were the means of sustenance in Eden, suggesting that in the best of conditions it was best that animals be spared. Moreover, Noah was but one party to God's "covenant of the rainbow" never to repeat the flood. The same promise was given to all living creatures, with no hint that their lives thereafter would depend on the vagaries of a human rather than a divine will.[8] To the contrary, on several occasions in the later books of the Old Testament, men are expressly directed to show kindness to the animals under their control. Thus, "You shall not muzzle an ox when it treads out the grain."[9] And, "A righteous man has regard for the life of his beast, but the mercy of the wicked is cruel."[10]

Perhaps the most persuasive evidence for restraint lies in the role-model of the good shepherd, often cited in both testaments. At one point, by report of the prophet Ezekiel, God becomes visibly annoyed with his chosen nation:

> Ho, shepherds of Israel who have been feeding yourselves! Should not shepherds feed the sheep? ... The weak you have not strengthened, the sick you have not healed, the crippled you have not bound up, the strayed you have not brought back, the lost you have not sought, and with force and harshness you have ruled them.[11]

God's own rule over his followers is often compared to the concern that a shepherd should have for his flock.[12] Designed to show that God stands to man as man does to the lower animals - a kind provider even if he has no duties to them - that simile would fail of its purpose if the shepherd could wholly disregard the welfare of his animals.

Aside from this figurative guide, the New Testament is conspicuously spare in its references to handling animals. For centuries the controlling precedent was taken to be Saint Paul's gloss on the proscription against muzzling the ox:

> Is it for oxen that God is concerned? Does he not speak entirely for our sake? It was written for our sake, because the plowman should plow in hope and the thresher thresh in hope of a share in the crop.[13]

Thus the thresher was to let his ox feed from the corn he worked, not for the good of the ox as such, but because a well-fed animal would yield him a larger return.

Perhaps this passage reveals less about God than about Paul, but it does point to a shift in sentiment which Christianity could be said to have condoned. For the Christian, the paramount practical concern became the condition and future of the immortal soul which he and other human beings possessed. Animals did not have immortal souls, nor were they repositories for human souls. Rejecting transmigration, Christianity developed a doctrine of rebirth which did not require that the soul inhabit a body in its next life. In the Christian world-view, then, animals were left without the one thing that had special value in itself. Their welfare, it seemed, was a good thing only as it was good for the human being with unconditional value.

The letter which contains Paul's reading of the Old Testament rule was written only a generation after Christ's death, when Christianity was still an infant faith without a theology to call its own. The distinction between man and the "lower" animals was to harden as the creed acquired the trappings of theory, but in such a way as to raise new questions about its real source. For the legacy of Greek philosophy was to exercise such a pervasive influence over Christian theology in its formative years that the distinction could as easily be traced back to Athens as to Jerusalem.

B) The Theologians on Animals

It is often said that in philosophy we are all still Platonists or Aristotelians of a sort. It might equally be said that in theology all roads lead back to Augustine or Aquinas. This is not entirely an accident. Saint Augustine was the first great Christian Platonist, Saint Thomas the greatest Christian Aristotelian. In Augustine's case, the path of influence passed through Neoplatonism, a more mystical variant of the original school which had emerged by the third century after Christ. By the latter half of the fourth century it could claim the young Augustine as a disciple. Augustine's celebrated conversion to Christianity was less a rejection than an assimilation of his philosophical roots. Building from an essentially Platonic dualistic view of human nature, he distinguished the body and the soul by their functions. The body was a tool subject to the direction of the soul. But if the human body was, from the standpoint of the soul, an article of use, then animals, which were bodies that lacked souls, must also have been instruments made available by God for our intelligent employment, without claims of their own against us.

The Augustinian position finds its polemical expression in his critique of the competing Manichean doctrine, which, on the premise that animals also had souls, would not allow killing them. Augustine cited the conduct of Christ as a lesson to the contrary.

> Christ himself shows that to refrain from the killing of animals and the destroying of plants is the height of superstition, for judging that there are no common rights between us and the beasts and trees, he sent the devils into a herd of swine and with a curse withered the tree on which he found no fruit.[14]

If Christ could use animals for his own purposes, then so apparently could we. Augustine's considered view, however, was tempered in two respects. First, he denied that animals were nothing more than instruments of men. As creatures made by God, they also possessed a good of their own that did not reduce to their value for us.[15] Second, the utility that they properly had for us was the use to which our intelligence might put them, not the convenience or inconvenience that they might be to our senses. Augustine did not hold that we were to treat animals according to our pleasure or displeasure with them.[16]

Benefiting from the later rediscovery of the Aristotelian corpus, Aquinas brought the Scholastic tradition to its full flower in the thirteenth century. His view of animals was more sophisticated and less sympathetic than that of Augustine. Every natural being which underwent development had an end or perfected state which God had created it to achieve. He made man, however, as a free and rational agent, with control over his actions. Man contained his end within himself; his life, that is, took its objective from his own design. Being neither free nor rational, an animal was merely a means to an end existing outside it (in the form of some purpose that a rational agent might have for it). Thus the nonhuman animal was ordered, by nature and providence, to the use of men.[17]

From Aquinas' perspective the Old Testament concern for animals took on a more characteristically Pauline hue. A man should avoid mistreating animals, not because this would be best for them, but because cruelty to animals might make him cruel toward other men, or because cruelty could be harmful to himself or someone else. Strictly understood, disinterested charity directed from a man to an animal was impossible, since there was no common fellowship between them.[18]

In its essentials this remains the prevailing view within the Catholic Church. Its implications for research in the life sciences have not gone unnoticed. Writing at the turn of this century, Father Joseph Rickaby, an English Catholic moral theorist, denied that the suffering of animals was an obstacle to biological inquiry.

> Brutes are as things in our regard: so far as they are useful to us, they exist for us, not for themselves; and we do right in using them unsparingly for our need and convenience, though not for our wantonness. If then any special case of pain to a brute creature be a fact of considerable value for observation in biological science or the medical art, no reasoned considerations of morality can stand in the way of man making the experiment, yet so that even in the quest of science he be mindful of mercy.[19]

Protestantism retained the thesis that man enjoys a rightful hegemony over the lower animals, but there are signs of a shift towards a "stewardship" interpretation of that role. John Calvin, the sixteenth century Reformation theologian, maintained that, when God placed animals "in subjection unto us, He did it with the condition that we should treat them gently."[20] They were brute beasts, to be sure, but for Calvin as for Augustine they were also creatures of God. Calvin went a step further than Augustine, however, in making this fact about animals a limitation on the use which man was authorized to make of them. But here man would seem to be less the sovereign of nature than a deputy whom God had appointed to manage His earthly estate. As the workmanship of God, every creature would still be subject to His ownership and control. Man would have the use of animals only in trust, with a duty of care over what in the last analysis remained in God's hands. A man was still worth more than any number of sparrows, yet "not one of them will fall to the ground without your Father's will."[21] Thus, as Karl Barth, the leading modern Protestant theologian, was to urge, we possess the right to use and sometimes to kill animals, but, given God's continuous oversight, only because He has so authorized it in order that we might live.[22]

The stewardship theory worked a change in the broadly political position of man within nature. From the secular sovereign with an absolute right to rule over animals, man became the deputy of a divine sovereign, the agent of a divine owner. This was not as such a theological change. The stewardship theory began to flourish, not by accident, in seventeenth and eighteenth century England, where a political theory of limited government had surfaced to lend it indirect support. Significantly the stewardship theory did not seek to overturn the existing philosophically inspired account of the distinction between men and animals. It remained the common currency of thought that men were free and rational agents while animals were not. That premise was not seriously challenged until the 1700s, and then on other grounds.

There have historically been a few distinguished Christian defenders of a position much closer to the Oriental view. Saint Francis, a generation before Aquinas, and Albert Schweitzer, a generation ago, both pressed for a principle of reverence toward evrey living thing, human or not. But their ideal has been received as just that - a norm perhaps for saints and something which all men should desire, but not thought to be binding on imperfect agents in less than ideal circumstances. In the absence of mainstream philosophical support, the reverence-for-life rule has not matched in intellectual authority the personal prestige that its practitioners have enjoyed as models of conduct.

C) Human and Animal Nature

Up to 1600, the philosophical mainstream was still Aristotelian. Using a much broader conception of the soul than the current one - though a vestige of it remains in our use of the term 'animate' - Aristotle distinguished living from nonliving beings by the presence or absence of a soul, or life-giving power, in some form. Its function might be nutrition, sensation, desire, locomotion, or thought. The first of these was found in plants but not the rest. All animals had sensation and desire as well, and most also had locomotion. Man alone had the power of thought along with all the others.[23] His advantage in this respect made him naturally suited to rule over others who lacked his special skill, and made animals natural slaves to man. Aristotle reached this conclusion by generalizing from phenomena already at work within the human species. Among men, those with the power of rational control over their actions exhibited an internal mastery of reason over desire and an external mastery - an aristocracy, or rule of the best - over those who, because they lacked the mental equipment to do more than minister to their bodily needs, required direction from others.[24]

Natural fitness implied that nature worked toward certain ends which together formed a master plan. This hypothesis, which had made the long-standing alliance between theology and philosophy possible, proved to be an impediment to the systematic study of nature. Purposes in nature were not directly observable, nor were they fit subjects for a rigorous quantitative study of empirical phenomena. The deeper significance of the seventeenth century Scientific Revolution lay not so much in its overthrow of Church authority in the empirical realm as in its discovery of a method and a subject matter (mechanics treated as a branch of physics) which made the hypothesis that nature had purposes dispensable. Nature became simply the sum of matter in motion, mathematically describable without reference to goals that phenomena might serve.

The philosophical foundations for the new world-view were supplied by Rene Descartes. Descartes recognized only two kinds of existence, material and mental.[b] Bodies were extended in space and time and divisible into parts, with properties of size, shape and weight. Minds contained beliefs, emotions and intentions, but none of the physical properties. Man was a composite being - the only one - with both a mind and a body.[25]

Animals did not fit comfortably into the Cartesian scheme. They obviously had bodies, but did they not also have sensations and desires? Descartes answered that in a sense they did, but that in that sense their behavior could be duplicated by a machine. Human behavior could not. In his use of language and thought a man revealed a capacity to respond to a given input in a variety of ways, whereas animals, like machines, would respond in only one, "according to the arrangements of their organs."[26]

For all their differences, the Aristotelian and Cartesian theories joined hands in making the activities which required reasoning the distinctive mark of humanity. Both defined man as a rational animal. That thesis was not put into question until the next century, when the British empiricist school cast a critical eye over what it took to be inflated claims for the power of reason, especially in discovering matters of fact. For the empiricist, reason alone, unassisted by experience, could give us no knowledge of the natural world. Moreover, such knowledge as we have has been acquired by patiently collecting and combining the data received by the senses. Reason itself, on this model, was less a creative than a calculative faculty which could never justifiably make claims that went beyond the strict boundaries of the data before it.

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[b] Cartesian dualism owed a great deal to Plato and Augustine, but it was not simply a return to older views. It was a new departure in at least two respects: (i) It did not treat bodies as instruments suited to the purposes that minds might have for them, and (ii) it made observable bodies the proper objects of scientific knowledge, not minds or the unseen mental essences of things.

The Scottish sceptic, David Hume, concurred with Descartes that the human mind was capable of creatively entertaining a variety of possible conclusions from a given body of experience. Inference, however, consisted in fixing upon one or at most a few of these possibilities. How was this process of selection to be justified? Why was it, in particular, that an increase in experience increased the tendency of the mind to focus on a single conclusion? It makes a difference whether we have seen the sun rise only once or a thousand times. But the difference, Hume argued, was nothing more than a habit of inference formed from repeated observations.[27] Yet dogs could do that much.

> 'Tis necessary in the first place, that there be some impression immediately present to their memory or senses, in order to be the foundation of their judgment. From the tone of voice the dog infers his master's anger, and foresees his own punishment. From a certain sensation affecting his smell, he judges his game not to be far distant from him.
>
> Secondly, the inference he draws from the present impression is built on experience, and on his observation of the conjunction of objects in past instances. As you vary the experience, he varies his reasoning. Make a beating follow upon one sign or motion for some time, and afterwards upon another; and he will successively draw different conclusions according to his most recent experience.[28]

The issue dividing Descartes and Hume survives, still unsettled, in current controversies over artificial intelligence and animal cognition. Recent decades have witnessed an explosion of empirical investigations into the behavior of nonhuman animals (ethology) and brain function in different species (comparative neuroanatomy).[29] Among these, various efforts to teach higher primates how to use a language have captured the public imagination. The chimp, Washoe, for example, was taught to use 132 standard ASL (American Sign Language) symbols over a two-year period.[30] The chimpanzee, Nim Chimsky, subject of an independent four-year experiment, managed to learn over one hundred ASL signs and use them with considerable facility.[31] Inferences drawn from such studies, however, encounter two philosophical obstacles. The first is methodological. The evidence for any conclusion must be behavioral: The chimps make the same gestures that a human deaf-mute would use in similar circumstances. To argue that they consciously use them in the same way is to assume a certain theory about the relation between bodily behavior and mental operations. But no consensus on the most satisfactory theory of the mind-body relation presently exists. (The same difficulty, incidentally, infects various efforts to use similarities in brain structure and function as evidence for similarities in thought.)

Assuming that such matters could be resolved, a greater conceptual hurdle would remain. What is it to use a language? What is the connection between language and reason, language and thought? Nim and Washoe were able to string names together in such a way as to communicate in a narrow range of circumstances. But language is a great deal more than a sequence of names.[32] It requires combining terms into well-formed sentences using rules of grammar and meaning. Linguistic mastery also includes the capacity to create novel sentences in situations not precisely like those already encountered and the resources to express thoughts in different modalities (as descriptions, questions, commands, and so on).[33] It would, finally, seem to require that one be able to recognize that what he says is true or false or uncertain.[34] By these standards of performance, the normal human child leaves Nim and Washoe far behind.

Although no one knows whether other primates will ever approach human beings in linguistic performance, it would be a mistake to rivet on that issue. Concluding a comprehensive survey of research in animal thought, Stephen Walker advises,

> Thought is a word rather than a thing, and we can use the word as we choose. If we use it to refer to psychological processes which cannot take place in the absence of human linguistic abilities, then animal thought is a conjunction of words that has no real meaning. But if we take it that some thinking can be done without words, and that a thought may be a mental disposition, an image, intention, or anticipation, then the question of whether this sort of thought can be said to occur in animals other than ourselves becomes genuine.[35]

Evidence is mounting that animals can recognize visual patterns, remember where their food is located, learn how to perform nonmechanical tasks, and foresee where a moving prey will eventually be positioned, even if they cannot master a language.[36]

Yet neither linguistic nor nonlinguistic findings will tell us all that we need to know. The moral issue is not simply whether animals have some and lack other abilities that human beings possess, but whether the differences between them make for differences in the respective ways that men and animals should be treated. Sometimes the differences matter, common sense might say, and sometimes they do not.

II. The Moral Questions

To ask how, if at all, animals should be used in testing, research, and education is to imply that there is only one question in need of an answer. Actually there are two, one preliminary to the other. Men like Aquinas and Dr. Baltimore have found it hard to see how animals raise a moral issue at all. Their doubts attach to what might be called the moral standing that an animal has. Is it the kind of being to which or about which men could possibly have duties? Although any answer to this question will have moral implications, the question itself is a conceptual one about the moral terms that we use and their appropriate referents. Our reasons for taking one side or another on it need not include any particular moral judgment. Whatever its resolution may be, a separable moral issue will remain: What constraints, if any, regulate our use of animals? One would expect these constraints to be weaker if animals lack standing but not necessarily absent altogether.

A) Moral Standing

The sex of an individual is a straightforward biological fact about him. That John Doe is a male is a simple matter of observation, difficult to determine only in rare instances. That John Doe is a United States citizen is equally a fact about him, but not one which can be established merely by looking at him. A certain directly observable fact - for example, that he was born in Chicago - would make him an American citizen, but the inference from the first description to the second requires an intermediate step. His place of birth determines his citizenship only because the norms of the American political community have connected the two facts. Had those norms been different - for example, making blood rather than birth the test of citizenship - the connection would be broken.

Citizenship is a role assigned to individuals by the norms of a political community. The citizen is distinguished by the rights that he enjoys and the duties which attach to his position. He has the right to vote; an alien would not. He has the duty to serve in the military; an alien would not. His rights and duties together give him the political standing which the alien lacks.

Political standing is typically conferred by law, but legal standing itself constitutes a broader classification. Legal standing goes to persons. "A person is such," Sir Frederick Pollock has said, "not because he is human, but because rights and responsibilities are ascribed to him. The person is the legal substance of which rights and duties are the attributes."[37] Citizens are of course persons, but so, too, are aliens. The person in law has standing to sue or be sued in a court of law. He is the individual who can have legal rights and duties, it being left to a particular legal system to specify precisely what his rights and duties are. Some individuals qualify as persons at law but not in all respects. A minor or a ward may have a right to life but not to enter into binding contracts, a duty to respect property but not to make reparation for what he has damaged. Finally, at the furthest remove from legal standing will lie individuals who are mere things at law, without rights or duties of their own, as a workhorse might be.

Modern moral theory operates under a "law conception" of ethics.[38] It takes particular human actions as its locus of assessment, judging them right (lawful) or wrong (unlawful) as they comply with or violate some universal principle of conduct. In this it departs from the classical theory of the virtues, which makes the agent and his character the unit of evaluation and does not attempt to reduce ethics to a system of rules. Under the law conception, moral standing also goes to persons, but with this difference: Moral standing is not conferred by any individual, institution, or community. From the moral point of view an agent counts as a person because of some characteristic that he has independent of any investiture. This is the chief reason why we may speak within the moral domain of the natural duties and the natural rights that a person can have. A legal sytem can of course recognize natural duties and rights, but once recognized, they cease to be natural.

Animals would have moral standing, in the sense specified[39], just in case they could be called persons; and they would be persons just in case they could have duties and/or rights. For obvious reasons, however, no one has ever argued that animals can have moral duties.[40] That would require that they freely choose to act among alternatives they judge to be right or wrong - a skill as demanding on the practical side as full-blown linguistic competence would be on the theoretical. The issue of standing, then, reduces to the question whether animals can have rights.

There are two broad theoretical families on the subject of rights. The first, sometimes called the "will" theory, would discourage efforts to attribute rights to animals. In its classical form, as given by Immanuel Kant, it would define a right as a capacity to obligate others to a duty.[41] Possession of a right carries with it an authorization to use coercion to enforce the correlative duty.[42] This in turn implies that the right-holder's capacity is a power of discretion, in cases where the duty may not be met, either to enforce it or waive it. A right, then, is something that one may choose to exercise or not. The choice itself will be an act of will.

H.L.A. Hart, the leading contemporary defender of the will theory, treats a right as a respected choice which gives the right-holder authority to control the actions of someone else. "It is," Hart adds,

> a very important feature of a moral right that the possessor of it is conceived as having a moral justification for limiting the freedom of another and that he has this justification not because the action he is entitled to

> require of another has some moral quality but simply because in the circumstances a certain distribution of human freedom will be maintained if he by his choice is allowed to determine how that other shall act.[43]

The will theory helps to avoid confusion between claims of right, which form only part of the set of moral judgments, and other, separable requirements to promote or secure some valued state of affairs (for instance, to assist someone in need). If rights were extended to the animal kingdom, Hart fears, the distinctive role which they play for agents endowed with discretion would be lost.[44] Lacking such power, animals could not be said to have waived or exercised the rights they had. But then all references to animal rights could simply be translated without residue into talk of human duties.

As an additional advantage, the will theory preserves a certain desirable symmetry between duties and rights. Since having a duty also requires that one possess the power to choose among alternatives, agents will have both rights and duties by virtue of the same characteristic. Freedom, too, seems to underlie both rights and duties, and it, too, arguably requires a will that animals lack.

Those who would assign rights to animals have embraced the alternative "interest" theory of what it means to have a right. A right, in their view, is a claim to the performance of a duty by someone else, but the right-holder need not be in a position or possess the competence to make this claim by an act of will. It is enough that he have interests of his own which can be represented, i.e., pressed by others on his behalf, in a normative forum.[45] His interests will include the things that are good for him and the things in which he "takes an interest" (selfish or not).[46] To have a right, then, will be simply to have interests which are affected by someone's doing his duty.

The interest theory surfaces in Peter Singer's Animal Liberation, the first contemporary theoretical statement of the case for animals. In that work Singer designates by the term 'right' any claim that an individual may enjoy to have its interests equally considered with those of others. It implies nothing more than a capacity for suffering, which both men and animals possess.[47] The modest measure of animal awareness that such a test demands has been one source of its appeal. It has not, however, been free of controversy. Some have objected that animals cannot have interests in the strict sense because they cannot have beliefs in the strict sense.[48] But even if this charge can be met, a more serious one remains. As stated, the interest theory shows only that having interests is a necessary condition for having rights, not that it is sufficient. Singer himself has since abandoned the attempt to show sufficiency and, accordingly, recanted his earlier references to the language of rights.

> I could easily have dispensed with it altogether. I think that the only right I ever attribute to animals is the "right" to equal consideration of interests, and anything that is expressed by talking of such a right could equally be expressed by the assertion that animals' interests ought to be given equal consideration with the interests of humans.[49]

He effectively acknowledges Hart's charge that the notion of a right has lost its distinctive function in this context. No longer referring to the discretionary control that one agent has over the conduct of another, it no longer adds anything to the straightforward language of duty.

There is a very general consideration, however, which appears to tell heavily against the will theory, if not entirely in favor of its competitor. It underlies a form of argument so ubiquitous in the animal rights literature that it deserves a name. The "consistency argument" is exemplified in the following passage from an essay on vegetarianism by Tom Regan. Rejecting rationality, freedom of choice, and self-consciousness as conditions for having a right to life, Regan adds,

> It is reasonably clear that not all human beings satisfy them. The severely mentally feeble, for example, fail to satisfy them. Accordingly, _if_ we want to insist that they have a right to life, then we cannot also maintain that they have it because they satisfy one or another of these conditions. Thus, _if_ we want to insist that they have an equal right to life, despite their failure to satisfy these conditions, we cannot consistently maintain that animals, because they fail to satisfy these conditions, therefore lack this right.
>
> Another possible ground is that of sentience, by which I understand the capacity to experience pleasure and pain. But this view, too, must encounter a familiar difficulty - namely, that it could not justify restricting the right _only_ to human beings.[50]

In short, in face of the fact that some human beings (infants, mental defectives, senile adults) evidently lack some such capacity as will, we cannot consistently hold (i) that that capacity is a condition of having a right and (ii) that all and only human beings have moral rights. Any less burdensome test, however, will presumably admit animals as possible right-holders.[51]

This reasoning is vulnerable on two counts. In the first place, it overlooks a significant difference between the incompetent human being and an animal. In all but a very few cases, the human being either will have or once had the capacity required. If rights do not imply _present_ possession of the qualifying condition, as is suggested by the way that we treat those who are handicapped only for a time, then babies who have yet to mature and others who have become incapacitated after a period of competence will still have rights. The animal, for all we know, has never met and will never meet this qualification. Second, the rare human being whose deficiency is complete over his lifespan is nevertheless differently situated from the animal. His condition is a _dis_ability, a loss of some skill he would normally be expected to have. The animal's condition is not disabling, even though he lacks the same skill. Thus the very fact that the human being has been deprived of his ability implies that he has been harmed; the very fact that he has failed to acquire it means that he is in need of help. The condition of the animal does not call for either inference.[52] This difference, to be sure, makes no mention of rights; yet it creates a special duty to meet the human need that would not extend to animals. Because the animal without a will has not lost what it was biologically programmed to possess, it "needs" a will only as a man might need to fly. In neither case does the condition give rise to a moral demand for assistance.

The consistency argument succeeds to this degree: It does show that the general reason for moral concern in the cases discussed cannot be limited to human beings. Other things being equal, the fact that a condition is harmful to or threatens harm to an individual, human or animal, creates a moral reason to intervene. That reason need not take the form of a duty owed to the victim, with the correlative right that this would entail. It need not always be a duty of any sort. We often reserve the highest approval for the good deed which, like the good Samaritan's, goes beyond what duty strictly requires.

There is a range of possible situations, beginning at one end with a strict prohibition against the cruel infliction of suffering, moving to a still powerful requirement to lend help where the agent alone is in a position to provide it for someone in great need, passing then to the milder requirements of charity and generosity where the agent can meet them without great personal sacrifice (even if others can do the same), and finally reaching, at the other extreme, the highly praised but not binding act of genuine self-sacrifice which distinguishes the moral saint from the run of men. The moral vernacular covers this spectrum with a single term. We say of the act in question that it was the "humane" thing to do, and sometimes that failure to do it would be inhumane.

The term itself refers to the agent, not the recipient. Humane treatment, following the Oxford English Dictionary, is "characterized by such behavior or disposition toward others as befits a man." The meaning given there, which dates back to the eighteenth century, applies to conduct "marked by sympathy with and consideration for the needs and distresses of others," where others can include both human beings and animals.

This does not mean that animals will generally command the same degree of affection and attention as other human beings. The attitude of sympathy, which is the psychological spring for humane treatment, consists in "feeling like" the object of sympathy, and the basis for this response must be a certain understanding of what it is like to be in the other's position. Other human beings are much more accessible in this respect, not only because they are structurally and functionally like us but because they can communicate their feelings to us in ways that animals can scarcely approach. In such areas as the capacity for experiencing pain, however, the differences across species are by no means so great as to make sympathetic identification impossible. Here the mark of the humane agent will be the extent to which his sympathy jumps the barrier between species.

There are differences among animals, too - in the capacities they have, the things they do, the relations they have with human agents - all of which affect the moral weight that humane considerations will have. A gorilla will gather more sympathy than a clam, not so much because it is more intelligent as because it exhibits a range of needs and emotional responses to those needs that is missing altogether in the clam, in which we can barely detect evidence of pain. Beasts of prey and rodent pests rarely elicit affection because their characteristic activities do not mark them as helpless and in need. Even within the same species, the quantum of regard that an animal may expect will rise with the social ties and responsibilities that human beings have developed with it.

As a possible recipient of humane treatment, the feral dog will stand to the pet dog much as the stranger among men does to an acquaintance. Familiarity breeds affection as well as contempt.

Each of the morally significant differences among animal recipients of humane treatment builds on an analogy to the human case. Thus, whatever the merits of the consistency argument on the score of rights, it finds a home here. We do not, it seems, put a brake on the humane treatment principle at the species border. Mary Midgley has put the point eloquently in another context:

> [Animals] can be in terrible need, and they can be brought into that need by human action. When they are, it is not obvious why the absence of close kinship, acquaintance or the admiration which is due to human rationality should entirely cancel the claim. Nor do we behave as if they obviously did so. Someone who sees an injured dog lying writhing in the road after being hit by a car may well think, not just that he will do something about it, but that he ought to. If he has hit it himself, the grounds for this will seem stronger. It is not obvious that his reasons for thinking like this are of a different kind from those that would arise if (like the Samaritan) he saw an injured human being. And he too may feel about equally justified in both cases in being late for his uncle's party.[53]

Humane treatment, it will appear, is the most commonly cited standard in federal legislation concerning animals. In view of its wide range of application, however, there is a temptation to dismiss it as a pious but essentially vacuous sentiment. The task for a theory of moral constraints is to determine whether this or some other standard can be made sufficiently precise to serve as a guide for legislation regulating the use of animals.

B) Moral Constraints

A rule which allowed an agent to do whatever he wished would not be a moral rule. Morality by its very nature operates as a check on the tendency to go wherever desire may lead us. The constraints it imposes on what we do can come from two directions. They can emerge, as it were, from the front of an action, looking ahead to its possible consequences, or from the side, placing certain restrictions even on the results we are permitted to promote.[54] From the front, the action that morally ought to be performed is the one with the best consequences. An agent succeeds in this objective just to the extent that his action produces as much benefit and as little harm as possible. From the side, conditions concerning such variables as the intention of the agent and the consent of the recipient may have to be met before a moral license to pursue best consequences is granted. If so, the fact that a lie will produce more benefit than the truth will not necessarily make it the right thing to do.

Moral theories divide according to the weight they give to one or the other kind of constraint. In its purest form, the forward-looking approach holds that an action or policy is right just in case it has better consequences, for everyone affected by it, than any available alternative. The language here is carefully drawn. 'Better', to begin with, does not mean 'morally better'. A good consequence is simply an outcome that is desirable from some point of view. If an action gives pleasure to someone, his enjoyment is a good thing; if it were to cause pain, his suffering would be a bad thing. One need not ask whether the pleasure or pain is morally fitting.

Ideally, intuition will play no part in determining the value of an outcome. One consequence will count as better than another if, after assigning positive numerical values to its good elements and negative values to its bad ones, the entire sum is greater than the summed values for the other result.[55]

Better for whom? The utilitarian principle, still the most influential formulation of the forward-looking approach, holds that actions and policies are to be evaluated by their effects, for good or ill, on everyone, not just the agent alone or some select group of individuals. "Everybody to count for one," according to Jeremy Bentham's rule, "nobody for more than one."[56] Between the agent's own good and the good of others, John Stuart Mill was to add, "utilitarianism requires him to be as strictly impartial as a disinterested and benevolent spectator."[57] The interests of each affected individual are to count equally, in that any experience with value, whoever has it, is to be included in the calculation of utilities and any two experiences which are alike but for their occurring in different individuals are to be given the same value. Among utilitarians, enjoyment is a good and suffering an evil if anything is; and so every animal with the capacity for such experiences will also count for one. Sentience suffices for possessing this value, even if it does not confer rights. "The question," Bentham once put it, "is not, Can they reason? , nor, Can they talk?, but, Can they suffer?"[58]

Because it extends the scope of moral concern to animals without committing itself to a vulnerable theory of animal rights, utilitarianism has become the theory of choice among those who would press for more constraints on our treatment of animals. Singer derives their credo - that all animals are equal - from the conception of equality just given.[59] If the principle of utility requires that we minimize suffering and if some kinds of human suffering are also found in animals, then to count the first and ignore the second would violate the canon of equality. It would make a simple difference of location, in one species rather than another, the basis for a distinction in value. Like racism, such "speciesism" enshrines an arbitrary preference for interests simply because of their location in some set of individuals.[60]

As a general moral principle, utilitarianism is subject to familiar objections, the most serious among them being that its standard of equality is much too weak to satisfy the demands of justice.[61] Since it only requires that individuals with interests be given the same consideration, but thereafter, in the summation of interests, allows the claims of any one individual to be overridden by the sheer weight of numbers on the other side, it seems to sanction a tyranny of the majority in morals which admits violations of individual rights. Within the domain of human relations this could be a fatal shortcoming. But it need not undermine the utilitarian case for animals if animals have doubtful standing as right-holders.

There may be an acceptable double standard in morals, consisting of a non-utilitarian principle for agents with standing as persons joined by a utilitarian rule for handling individuals with interests but not rights.[62] The use of different rules for different kinds of individual is already a well-established procedure. Rules which would be objectionably paternalistic if applied to adults are admissible if restricted to children. The only dangers are that both standards might hold for the same individual, giving inconsistent directions, or that the differences between the two classes of individual might turn out to be arbitrary. This does not seem to be the case either for the adult-child or the man-animal distinction.

The device of a dual standard is just what laymen seem to use in explaining the sharp difference in the constraints governing our treatment of animals and human beings as experimental subjects. For animals the standard is humane treatment, which forbids inflicting unnecessary suffering but otherwise allows experiments that harm and even kill the animal. That same rule, proposed for human subjects, would be dismissed out of hand. There are many experiments, including much medical research into the major diseases, in which perfectly reliable results can only be obtained by doing to a human being what we now do to an animal. Nevertheless, in the absence of the subject's informed consent, they are absolutely impermissible, no matter how beneficial (for everyone concerned) the consequence might be. They would violate the rights of the human subject.

The proscription against unnecessary suffering is best understood as a corollary of the principle of utility. Since suffering is a bad consequence, there is an initial utilitarian onus against behavior which would produce it. Such treatment calls for justification. To meet this burden, a bare appeal to some offsetting good consequence will not be sufficient. The principle of utility, as formulated, is comparative. It requires that an action or policy have better consequences than any available alternative. Among the alternatives will be uses which do not involve animal suffering. If just one of them has consequences at least as good as or better than the one proposed, the suffering will be unnecessary. Other things being equal, then, it should prove harder to establish necessity than the contrary, since the one must rule out all the alternatives while the other need find only one.

Necessity is a relation between a means (an action or policy) and an end (its objective). An inquiry into necessity can be restricted or unrestricted. If restricted, it will take the end as given - that is, not subject to evaluation - and ask only whether the course of action suggested is an indispensable means to that end. To take as an example an LD50 test for toxicity which uses forty rats as subjects, if no alternative procedure using fewer or no rats could get the same results with the same reliability, that test would be necessary in the restricted sense. In the case of unrestricted necessity, the end in turn will be open to assessment on utilitarian grounds. Here two additional questions can be introduced: (i) How likely is the objective to be met, in comparison with other possible goals? The lower the chance of its being realized, the higher the chance that a more beneficial alternative exists. Thus if the LD50 test should prove to yield unreliable results, its necessity in the unrestricted sense would be open to challenge. (ii) Assuming that the objective will be met, how beneficial will it be? Suppose, for instance, that an LD50 test were to be run on a new brake fluid not significantly different from those already on the market. This may prove unnecessary in the sense that the cost in suffering might better be borne in checking some other potentially carcinogenic chemical.

Of the two kinds of necessity, unrestricted necessity will be the more difficult to prove, because it always includes restricted necessity and more. Thus if we wished to lay down a stringent standard of necessity, one which let fewer procedures through, we would insist that they be necessary in the unrestricted sense. Moreover, since the necessity for a procedure is a more difficult matter to establish than the possibility of substituting for it, we would make the former the conclusion to be defended and place the burden of proof on those who would use the procedure.

A more lenient test would invert these priorities. It would operate on the presumption that the procedure was necessary unless shown otherwise and thus effectively make the absence of necessity the matter in need of demonstration. It would not expect the user to show beforehand that no other alternative was available.

For reasons which will become clear, the existing standard of necessity is comparatively lenient.

Nonutilitarian positions on the use of animals have one feature in common:

Although virtually none of them ignores consequences, they unite in denying that a course of action can be justified wholly by appeal to the value of its consequences.[63] This leaves room for substantial variation, the differences among them traceable to the considerations they would add in order to complete a moral assessment.

Ironically, both extremes in the animal treatment debate are nonutilitarian. The hard line against the claims of animals builds from the premise that they lack moral standing. Without rights they cannot be recipients of a duty owed to them. On some theories of value, moreover, enjoyment does not count as a good thing in itself, nor is suffering in itself an evil. Kant, for example, thought that the only unconditional good was a will whose choices were undetermined by desire for enjoyment or fear of suffering (in the form of punishment).[64] Not having a will, animals could not have this value. Morally, they were indistinguishable from inanimate tools - mere means to be used for the purposes of beings who do have a will. Like Aquinas, however, Kant did acknowledge an indirect duty of kindness, given that "tender feelings toward dumb animals develop humane feelings toward mankind."[65]

The indirect duty theory stumbles in the attempt to explain why there should be any empirical connection at all between an agent's feelings for animals and his feelings for men. He must see some similarity in the objects of the two sentiments if one is to influence the other; yet the theory says that there is no such likeness in reality. Thus either his motive is proof by itself that we have a direct moral interest in animals, in which case the theory is mistaken; or the theory is correct and the agent has misunderstood it, in which case he will be free, once educated in the theory, to abuse animals without fear that this will tempt him to abuse human beings. Kant cannot have it both ways: He cannot require agents to act on a belief that his own theory alleges to be false.[66]

The Kantian position could be turned on its head if animals had moral standing after all. In The Case for Animal Rights, Tom Regan gives the most cogent defense to date for that minority view. Regan begins by conceding that animals are not moral agents. Since they are unable to choose freely among impartially determined moral alternatives, they cannot have any moral duties. At least some animals, however, are "subjects-of-a-life". They have beliefs and desires, memory and a sense of the future, preferences, an identity over time, and an individual welfare of their own.[67] In these respects they are indistinguishable from human infants and mental defectives, who also fail to qualify as moral agents. Nevertheless, subjects-of-a-life possess an inherent value, independent of the value that their experiences may have, which gives them standing as "moral patients" - that is, as individuals on the receiving end of the right and wrong actions of moral agents.[68] They have this value equally, and equally with moral agents.[69] Inherent value in turn gives them a claim, or right, to certain treatment.

Regan's reasoning so far is designed to establish standing for animals. Since it relies heavily on the consistency argument, it could be challendged on that ground. But the differences between human beings and animals also appear to undermine Regan's theory of constraints. His major thesis is that, as moral patients, animals enjoy a presumptive right not to be harmed. He considers this principle a radical alternative to utilitarianism. But at least initially, once the reference to rights is filtered out, the utilitarian might find Regan's theory quite congenial. Harm to animals is a bad consequence on his position, and so it would be wrong, in the absence of an overriding consideration, to harm them. The conflict between the two theories must lie, therefore, in the kind of justification that each would permit to overturn this presumption. Regan offers two such reasons. By the first, when we must choose between harm to a few and harm to many, and each affected individual would be harmed in a comparable way, then we ought to override the rights of the few.[70] In these cases, however, Regan acknowledges that the utilitarian commitment to minimize suffering would have the same result.

By the second principle, when we must choose between harm to a few and harm to many, and the affected few (taken as individuals) would be worse off if harmed than the many (again, as individuals) would be if they were harmed, then we ought to override the rights of the many.[71] This "worse-off" rule parts company with utilitarianism in setting aggregate consequences aside. It also has an implication that will disturb Regan. In cases where it comes down to a hard choice between animal and human interests - biomedical research, for instance - the worse-off rule will weigh heavily against the interests of animals. For one thing, it will shut off the standard appeal to the massive numbers of animals sacrificed to the ends of research. As long as the harm to affected individuals is different, the numbers will not count.

More troubling still, the harm to men and animals in such cases will almost always in fact be different - and greater in the human victim. The reason can be traced to the connection between disease and disability. Consider the case of two human beings suffering from a bone cancer that requires amputation of a limb. Between them, the one who has lost the greater ability will have suffered the most harm. Even if they both lose a left leg, it will make an enormous difference that one of them is a professional running back, the other a sedentary executive. Both are badly off, but the football player much more so. Now if medical research were faced with the alternatives that an animal in the laboratory or a human being in the world should contract this same disease, there might be nothing to distinguish the two on the score of pain and bodily dysfunction; but the human will incur the greater disability simply because he has a great many more abilities to lose. These abilities will be missing in the animal but not lost, and so their absence will not make it worse off.

In view of this possibility, it is surprising to find that Regan calls for a blanket prohibition against the harmful use of animals in toxicity testing and research. That conclusion would follow only if his two rules for defeating the right not to be harmed could never be successfully invoked in these areas. Regan is apparently drawn to this result by a side-constraint which he attaches to the rules: They hold only for harms suffered by innocent victims. Animals are always innocent, in the sense which Regan gives to that term.[72] But human patients will be, too, and at least sometimes human agents will also be. Regan would have to show that these occasions can never arise in testing, research, or education, or that, if they do, the human agent/patient never faces the greater harm. Regan's analysis fails to meet either burden.

This difficulty aside, Regan's theory can be read as holding, first, that the necessity standard cannot be applied until the innocence of all parties has been established, and second, that when it does apply, the worse-off rule should replace the utility principle in cases where they diverge. His challenge would be met by showing, first, that the notion of innocence, with its judicial implications, has no place here if animals lack standing as right-holders, and second, that the worse-off rule is not necessarily a preferable animal protection clause to the utilitarian principle.

III. Moral Bases for Federal Legislation Protecting Animals

Although the relation between legal and moral principles is currently a controversial matter[73], one would expect in general that legislation imply - without necessarily aritculating, much less encoding - a consistent and defensible moral position. Even those who maintain that governments have no business legislating morality would grant that laws which fail to meet the condition stated are unstable and ripe for repeal.[74] The post-war judicial and legislative record on civil rights is a case in point. The massive reshaping of existing laws over the past two generations required, to be sure, a strictly legal justification; but it would be fanciful to deny that the impetus for these changes came from the widely held conviction that the laws as they stood were morally indefensible.

Legal and moral concerns are in this sense connected. It would be a mistake, however, to draw the connection too tightly. The legal rationale for federal laws protecting animals has frequently been the interstate commerce clause of the Constitution. In order to use that principle, Congress has been obliged to cite some economic interest which would be served by a particular piece of legislation. In purely economic contexts, animals will appear as property, their interests judged merely as means to human ends. One might hastily conclude from this that the reasons for a law were really economic, when all that strictly follows is that these were the legally sustainable grounds. Other language in a statute - a humane treatment requirement, for example - might be a more accurate barometer of Congressional intent, even though, if it stood alone, it would be insufficient to sustain the law. Its very presence would be revealing; for if Congress had had no direct concern for animal interests, the reference would be otiose. It would not be necessary to add that treatment already dictated by economic considerations be humane as well.

A) Legislation Protecting Animals as Individuals

Legal sanctions against cruelty to animals were introduced at the state level in the nineteenth century. The first such law, passed by the New York state legislature in 1828, made it a misdemeanor offense to "maliciously and cruelly beat or torture" an animal.[75] Over the next century, virtually every state followed suit. The first federal statute to protect animals, the Twenty-eight Hour Law of 1873, was enacted at the height of this trend. In the decade before and the decade after its passage, twenty-two states implemented anti-cruelty laws.[76]

Some state laws extended to the transportation as well as handling of animals, but these provisions could not reach beyond state borders into interstate commerce. By the early 1870s, abuses in cross-country rail transportation of livestock had become public knowledge. The animals were carried for days, often without food or water, in cars so overcrowded taht they were forced to remain standing. Losses in life, health, or at least weight were enormous.

The new law was expressly designed "to prevent cruelty to animals while in transit by railroad" or other means.[77] It limited the period during which animals could be continuously confined to twenty-eight hours. After that, the carrier was required to unload them into facilities with adequate provision for food, water, and rest, and to let them remain there for at least five hours. Exceptions were granted for bad weather or accidents. Apparently as an inducement to improve their own facilities, the act exempted carriers whose cars already contained adequate provision for the animals. Responsibility for complying with the time provisions fell on the carrier, but the expenses of care during the rest period were to be borne by the owner. Should he fail to comply, the carrier was to take over, recouping his expenses with a lien permitted by the law.

Given that the federal authority to regulate interstate commerce had not yet been clarified, opponents questioned whether the new legislation was constitutional. Surprisingly, although the railroad and livestock industries had great political influence at the time, they did not apparently challenge the law on grounds of economic hardship. In theory it should have at least reduced their losses in transit. The Senate sponsor of the bill, Senator Wilson of Ohio, nevertheless pushed for passage on two different grounds.

> It is very easy from the facts to understand the cruelty, the barbarity, and the brutal inhumanity practised upon these herds in transit. It is not only disgraceful to American civilization, but it is detrimental to the health of the people of those cities who must eat the flesh of these animals. They are unawares taking into their stomachs the seeds of disease and death.[78]

The act, then, was to protect both the animal in transit (from cruel and inhumane treatment) and the public which would consume it (from a threat to health).

Even if the Twenty-eight Hour Law advanced human interests by helping to insure that livestock were better products for consumption, the animals were clearly more than means to this end; they were the intended direct beneficiaries of the new legal obligation. This is not to say that the owners and carriers now owed a duty _to them_. Failure to comply with the act did not give rise to a claim in or on behalf of an animal. The sanction imposed was a civil fine, not a right to sue in the injured party. It would be a misreading of the law, therefore, to infer that it recognized a right against cruelty in the animal; it strictly forbade cruelty, regardless of rights, adding a legal sanction to back one form of the broad moral demand for humane treatment.

Senator Wilson's concern for the public health was farsighted. It was not until the turn of the century that public interest was to shift from transportation to the food production process itself, and the initial locus of concern was the hazard to health created by conditions there. Nationwide exposure of abuses in the meat packing industry served as the chief catalyst for the Meat Inspection and the Pure Food and Drug Acts. There was no matching attention to the suffering that an animal faced at the slaughterhouse stage, perhaps because the technology did not then exist to make its death easier. To render a cow unconscious before killing it, a skilled laborer had to deliver a precisely targeted blow to its skull with a long sledgehammer. Should he miss, the still conscious animal, in great pain, would become a much more difficult moving target. Often several blows would be needed. Over the years more reliable methods - the captive-bolt pistol, electric shock, the CO_2 tunnel - were developed; but as late as the 1950s, no law mandated that they be used. In 1958 Congress acted "to establish the use of humane methods of slaughter of livestock and poultry as the policy of the United States."[79]

The Federal Humane Slaughter Act was premissed on Congressional findings that

> The use of humane methods in the slaughter of livestock prevents needless suffering; results in safer and better working conditions for persons engaged in the slaughtering industry; brings about improvement of products and economies in slaughtering operations; and produces other benefits for producers, processors, and consumers which tend to expedite an orderly flow of lovesotck and livestock products in interstate and foreign commerce.[80]

A humane method, within the meaning of the Act, was

> 1)a method whereby the animal is rendered insensible to pain by mechanical, electrical, chemical or other means that is rapid and effective, before being shackled, hoisted, thrown, cast or cut; or
> 2)a method in accordance with ritual requirements of the Jewish faith or any other religious faith whereby the animal suffers loss of consciousness by anemia of the brain caused by the simultaneous and instantaneous severance of the carotid arteries with a sharp instrument.[81]

To resolve further disputes over what might constitute a humane method, the law authorized the Secretary of Agriculture to study and support research into slaughtering methods that were both practicable and humane, and then to designate which ones did or did not conform to the legislated policy. Failure to comply with the terms of the act brought no civil or criminal sanction. The slaughterer was not in fact under any direct obligation to change the procedures he used. The law drew its power to coerce from the duty that it imposed upon all governmental agencies to take the new policy into consideration when administering government procurement and price support programs. Since these programs touched four out of every five meat processors in the country, the effective sanction for the act became the fear of large business losses.

The bill in final form was an effort to mediate among several competing interests. The meat packing industry opposed it for economic reasons, and many believed that it would force a sharp increase in meat prices. Some executive agencies had reservations. The Department of Defense argued that it would interfere with procurement in some situations, and the Department of Health, Education, and Welfare voiced fears that some of the methods approved as humane might nevertheless tend to adulterate the meat product.[82] Finally, Jewish leaders rejected early versions of the bill which made no allowance for the ritual requirement in their tradition that an animal be conscious when killed.

As might be expected, the humane societies were the strongest supporters of the new law, but they were joined, among others, by the Meat Cutters and Butcher Workmen's union.[83]

The various ways in which these interests surfaced in the law furnish valuable clues to federal policy on handling animals. To begin with, the economic argument did not as such succeed. Congress found on the facts that humane methods would prove efficient in the long run, even allowing for costs of conversion. Thus it did not feel forced to choose between humane and economic objectives. Yet as in the Twenty-eight Hour Law, this left undetermined what Congress would decide if faced with a genuine collision between reasons of economic impact and humane treatment - for example, in "factory farming" procedures for raising livestock and poultry.[84] Both bills directly protect the interests of animals but they permit no inference to the effect that these interests would always prevail over strong and squarely conflicting economic claims.

In other respects, however, the Humane Slaughter Act provides a very clear formulation of federal policy. First of all, it introduces the necessity standard as the test of humane treatment. Other things being equal, a slaughtering method is inhumane if it inflicts needless suffering. Within the context of the act, suffering would be unnecessary if there were an available alternative which i)had the same result (the death of the animal), ii) caused less pain to the animal, and iii) had no significantly greater cost of some other kind. As structured, the test would allow for circumstances - national emergencies, the exigencies of defense purchasing - in which methods no ordinarily necessary might become so. These exceptions were included in the law. It would also take cognizance of HEW concerns about adulteration. It is an empirical question whether a given method has such costs, and an answer to it could presumably come from the further research into humane methods provided for in the law.

On the delicate issue of ritual slaughter, the bill appears at first glance only to incorporate confusion. In section 2, the Jewish orthodox procedure counts among the acceptable humane methods. Yet in section 6, ritual slaughter is exempted from the provisions of the act. Section 2 would seem to have made that unnecessary. A distinction will help explain this curious combination.

The ritual practice at the point of killing humane, since the animal will lose consciousness almost instantly. Before that, however, when being "hoisted, shackled, thrown, cast, or cut", it will still be conscious. The hoisting process can and often does break the pelvic or leg bones of the animal, putting it in great pain for the few minutes before the final cut is made.[85] The exemption was to allow for that part of the ritual slaughtering procedure.

There are three possible justifications for the exemption. The first would be that a religious requirement is really a special case of necessity. The demands of scripture do not change the fact that causally efficacious alternatives exist; but they do impose a practical necessity that effectively prevents the orthodox practitioner from using these alternatives. If this were the reasoning of Congress, however, the ritual slaughter would have been covered under a broader conception of necessity rather than as an exception to an existing rule. A second possibility would be to treat the case as one in which a basic human right stands in the way of securing the animal interest. The language of section 6 favors this reading of Congressional intent:

> Nothing in this chapter shall be construed to prohibit, abridge, or in any way hinder the religious freedom of any person or group.

Looking to the standard from this point of view, other things are not equal - and so, the normal utilitarian calculus cannot be made - when a measure to protect the interests of animals infringes a basic human right. Put another way, although benefits to man do not necessarily override benefits to animals, the rights of man do. A qualification of this kind will surface once more in the research area.

Common sense suggests a third explanation, but one which is a much less likely gauge of Congressional intent. Since the ritual slaughterer allows the animal to suffer for reasons of religious conscience alone, he lacks the cruel and malicious intent that we usually associate with inhumane treatment. Thus he is not the sort of person that morality or the law aims to discourage.

Notice, however, that both the Twenty-eight Hour and the Humane Slaughter Acts make a certain form of conduct inhumane, regardless of the intention behind it. The reason is clear. To insist that malicious intent be established would seriously reduce the prospects for vigorous enforcement of the legal requirement. The Horse Protection Act of 1970 serves as an object lesson in this regard.[86] The Tennessee Walking Horse is a show horse known for the distinctively elegant, high-stepping gait which comes from inbreeding and extensive training. Such horses have great value as potential prize-winners in competition and as breeding stock for future champions. After the second World War, however, trainers discovered that they could artificially reproduce the characteristic gait by "soring" a horse. If a blistering agent, nail, heavy chain, or injection were applied to its front feet, it would walk in the desired way to avoid placing weight on the affected area.[87] The Horse Protection Law was enacted to put a stop to this practice. It was premissed on these Congressional findings:

1) that the practice of soring horses for the purposes of affecting their natural gait is cruel and inhumane treatment of such animals;
2) that the movement of sored horses in commerce adversely affects and burdens such commerce;
3) that horses which are sored compete unfairly with horses moved in commerce which are not sored.[88]

Like its predecessors, the law expressed Congress' customary dual but independent concern for animal and human interests. Soring was prohibited both as an injury to the horse and a fraud against the human consumer who unwittingly overvalued it. The original legislation, however, had virtually no impact on the practice it was intended to stop. It imposed a duty to comply, enforceable by periodic inspections, on horse show exhibitors and directors, with civil sanctions for knowing and criminal penalties for willful violations. It proved especially difficult, however, to establish that soring had occurred, still more that it had been done intentionally. Six years later, Congress made wholesale revisions in the Act to remedy these deficiencies. Among other changes, any pain in walking, other than from therapeutic treatment, was to be considered a result of soring, and soring was to be judged cruel and inhumane without regard to its purpose. Various failures to take active steps to prevent exhibition of sored horses now became infringements of the law, and criminal liability was imposed for knowing but not necessarily willful violations.[89] The direction of all these amendments was clear: Congress had come to worry less about detecting or altering intentions in offenders and more about eliminating the practice as such. The amendments mark a return to the general trend of federal policy, which has been to make punishment for malicious behavior a means to rather than an end independent of animal protection.

Although none of the foregoing legislation touched the areas of research and experimentation, the principles laid down there find their natural home in the Laboratory Animal Welfare Act of 1966.[90] Placed in perspective, the terms of this enactment were quite modest. Four years earlier, Senators Clark and Neuberger and Representatives Griffiths and Moulding and introduced bills which would have imported the substance of the landmark British Cruelty to Animals Act of 1876.[91] The preamble to the Moulding bill attests to their sweeping character:

> It is declared to be the policy of the United States that animals used in experiments, tests, the teaching of scientific methods and techniques, and the production of medical and pharmaceutical materials, shall be spared avoidable pain, stress, discomfort and fear, that they shall be used only when no other

> feasible and satisfactory method can be used to obtain necessary scientific information for the cure of disease, alleviation of suffering, prolongation of life, or for military requirements, that the number of animals used for these purposes shall be reduced as far as possible, and that all animals so used shall be comfortably housed, well fed, and humanely treated.[92]

All of the bills died in committee, unable to survive united opposition from executive agencies and the scientific community.[93] At that time, no proposal to regulate the use of animals in research, testing, or education had much chance of success.

Accordingly, efforts to protect animals shifted to the antecedents of experimentation. They gathered momentum from media coverage of black-market operations that sold stolen pets to auctions which supplied the nation's laboratories. If Congress could not regulate the experiments themselves, it could regulate "the transportation, sale, and handling of dogs, cats, and certain other animals intended to be used for purposes of research or experimentation." This was necessary

> to protect the owners of dogs and cats from theft of such pets, to prevent the sale or use of dogs and cats which have been stolen, and to insure that certain animals intended for use in research facilities are provided humane care and treatment.[94]

The Laboratory Animal Welfare Act was to meet these objectives by authorizing the Secretary of Agriculture to license dealers supplying animals for research, to require that research facilities purchase their animals only from licensed dealers, and to promulgate standards of humane treatment governing the handling and transfer of animals by both dealers and research facilities.[95] These provisions were not to be construed, however,

> as authorizing the Secretary to prescribe standards for the handling, care, or treatment of animals during actual research or experimentation by a research facility as determined by such research facility.[96]

Because the law gives first notice to the property rights of pet owners, it provides fodder for a somewhat cynical review.[97] The animals are mere things, the language seems to imply, not judged worthy of protection on their own. Yet if stolen animals were really no different from stolen automobiles, it would be a mystery why the humane treatment sections were included in the legislation. Mistreated dogs could just as well have been recognized as damaged goods, for which the dealer or laboratory should furnish compensation but nothing more. If the dog were killed or lost, it would be enough, on the "mere property" reading, to give the owner its replacement value. And as a criminal act, the theft itself could have been classified as a straightforward case of petty larceny. The original act and the direction taken by amendments confirm that something more than compensation or punishment was at stake. In 1970 its protections were extended to all warm-blooded animals, domestic or not, and to uses other than research (for example, exhibitions) where ownership claims were not in dispute. By 1976 intermediate handlers (notably air cargo carriers) were brought under its jurisdiction, and animal fighting ventures were outlawed. By that time, humane treatment had assumed first position in the statement of Congressional objectives and the "pet" clause was a more distant third.[98] The property-right issue had become but one of several Congressional concerns.

The major battle in the legislative process was fought over the legal territory to be held by animals and animal users, respectively. With the deflated trial balloons of 1962 in mind, Congress was sensitive to the claims of the research community. Regulation was to cover the care and handling of animals before and after experimentation, but not "during actual research", and the laboratory itself was to decide where these lines should be drawn. Since most laboratories had only a single facility to serve as the site for all three stages and since many experiments had time frames measured in months or years, the borders of the "actual research" domain could extend quite far.

In the 1970 Amendment, Congress went one small regulatory step further. Section 13 was altered in two ways. First, humane standards were now to include "the appropriate use of anesthetic, analgesic or tranquilizing drugs, when such use would be proper in the opinion of the attending veterinarian of such research facilities." This change applied only to handling before and after research. But a second addition touched the experiment itself. To the original exemption for actual research, Congress attached this proviso:

> That the Secretary shall require, at least annually, every research facility to show that professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, during experimentation are being followed by the research facility during actual research or experimentation.[99]

Explaining why the change was made, the House Committee Report on the legislation noted,

> The bill in no manner authorizes the disruption or interference with scientific research or experimentation. Under this bill the research scientist still holds the key to the laboratory door. This committee and the Congress, however, expect that the work that's done behind that laboratory door will be done with compassion and with care.[1]

Thus, though the laboratory must certify to an external authority that it has complied with humane standards, the substance of the standards is to be drawn from the scientific community itself. To the extent that this is regulation, it is self-imposed.

What justifies the immunity given to the research enterprise? As in the ritual slaughter case, there appear to be three possibilities. The first would be that the scientist does not maliciously inflict suffering in the course of an experiment. He has (intellectual) reasons for doing what he does, and they suffice to remove his actions from the category of the wantonly cruel. Such reasoning may possibly underlie the exemption given to scientific research in many state anti-cruelty laws[2]; but it is ill-suited to the federal context, which looks to overt conduct as the test of lawful treatment. The humane standards to be promulgated under the Laboratory Animal Welfare Act are to include provision for "housing, feeding, watering, sanitation, ventilation, shelter from extremes of weather and temperature, separation by species, and adequate veterinary care."[3] Good intentions alone would not presumably justify failure to meet them.

The second ground would be the condition of necessity itself, which, though not part of the language of the Act, could be inferred from other legislation. Unlike the ritual slaughter requirement, however, the necessity found in the scientific context is the same causal necessity connecting means and ends that is in question elsewhere. The difference is in the benefit rather than the cost side of the moral ledger. The most common defense of harmful animal research and testing is that only in this way could we have secured the enormously valuable results of modern science - most prominent among them the long list of breakthroughs against the major diseases in this century. The necessary connection to a very great good is what justifies these experiments. Yet this would not justify exempting all of them from regulation. The argument is really that animal use of this kind satisfies the necessity condition - a quite different matter. It is one thing to say that research and testing protocols cannot be touched by regulation and another to say that, because it complies with the existing legal standard, it should not be disrupted. Furthermore, once the defender of research and testing chooses to argue from its benefits, he virtually surrenders the case for a blanket immunity from regulation. For he has in effect conceded that research and testing are merely means to other desirable ends; and given the differences in utility among the various forms of research - from pure to applied, and, within the latter, from basic cancer research to toxicity testing for cosmetics - he will have no way to guarantee that all of them are so useful that they overcome the strike against them. Nor will it help much to add that some great discoveries have been serendipitous side-effects of experiments with other objectives. The antecedent probability that such happy accidents will occur is low - they would not otherwise be accidents - and so their discounted utility will be correspondingly low.

The last and perhaps the most secure ground for an exemption would be the basic right to acquire knowledge free from external interference. But this reasoning has its limits, too. Opponents of regulation will sometimes say that the history of science is the story of a liberation, long in coming, from the rearguard actions of the Church and from the intrusion of subjective value judgments into investigations that are properly "value-free". These claims have an element of truth, but they are beside the point. They point is that the scientist has always recognized that there are some things he (morally) cannot do. He cannot, for instance, abuse a human subject in the pursuit of knowledge. Thus the right of inquiry cannot be pressed as an absolute title to operate as one wishes. Possibly, then, the argument is that, though the necessity standard does constrain the scientist in his use of animals for experimentation, he and not the layman has the right to decide when that condition has or has not been met. In the research context, it will frequently be impossible to determine whether allowing an animal to suffer is necessary unless one is possessed of complex empirical knowledge and a sophisticated feel for the methodology of science. Here again, the defense holds to a point, but it creates no bar to regulation. It shows only that any such review should be done by one's peers. Even if this were not so, even if nothing could override his right to decide, it would remain only that - a right to choose which research or testing program he would try to pursue. It would not follow that he had a right to funding for the investigation he desired, still less that he should be given the means to conduct it with no conditions attached. The Laboratory Animal Welfare Act leaves it open what Congress might decide if it should find that the work performed behind the laboratory door was not done with "compassion and care."

B) Legislation Protecting Animal Species

The laws protecting animals on their way to slaughter or the cancer lab certainly do not protect their lives. But some legislation has gone this far. The humane treatment laws regulate abuses toward animals already under the control of man. But even in their natural environment, animals can become victims of human activity. We now know that man himself is one of the principal agents of biological extinction, that the species he threatens to destroy often perform critical ecological functions in the world they inhabit, and that loss of these functions can adversely affect larger human as well as nonhuman interests. But since the relations between man and beast in these areas are less direct and further removed from the morally paradigmatic man-to-man relation, they are harder to catch within the moral net.[4] The relevant principles are less obvious than and less obviously connected with the standard of humane treatment.

Congress entered the field with the Marine Mammal Protection Act of 1972.[5] It found that certain species and population stocks of marine mammals, including whales, porpoises, seals, and polar bears, were in danger of extinction or severe depletion from indiscriminate hunting and fishing practices. Not all of it was deliberate. The threat to dolphins, for instance, came from their entering and finding themselves unable to escape the large nets set by tuna fishermen. To reduce these and other losses, Congress declared that, until such time as administrative regulations could be laid down, there was to be a moratorium on the "taking" of marine mammals.[6] Taking included hunting, harassing, capturing, or killing.

The immediate objective was to keep the population of affected species from falling below a sustainable level. That would preserve a natural resource thought to have aesthetic and recreational as well as economic value.[7] Careful management would also restore a measure of balance to the marine ecosystem. On the other hand, regulation had its unwelcome side-effect in the threat that it presented to the livelihood of human beings at work in these areas. The four years of debate and multiple revision that the bill underwent bear witness to the strength of such opposing interests as the offshore commercial fishing industry. To relieve their distresses, exceptions were made in the final version for those who would suffer undue economic hardship, for Alaskan natives using traditional hunting practices for subsistence, and for scientists removing animals for research purposes.[8] The Act also permitted takings in the course of commercial fishing, as long as these were kept to minimal levels.

The numbers taken were not, however, the only subject of Congressional concern. The callous methods employed in doing it were also disconcerting. Accordingly, such taking as the law allowed by way of exception was to be done humanely, with "the least degree of pain and suffering practicable to the animal."[9] This did not mean that the Act was really a humane treatment law. The general rule of conduct which it sought to encourage was merely to leave the animals alone in their natural habitat, once balance was restored; and 'humane' is not the appropriate accolade for simple noninterference.

When it became law, the Marine Mammal Protection Act was the strongest and most comprehensive legislation of its kind in the world.[10] But when paired with the Endangered Species Act, passed one year later,[11] it bears the marks of a prologue. The two laws are structurally much alike, but the second goes farther in virtually every respect. Congress found that over one hundred species of fish and wildlife in the United States, and over three hundred abroad were close to extinction from human hunting and from unchecked economic development that had destroyed their natural habitat. The Congressional interest in reversing this trend went beyond the aesthetic or economic; many of these animals were thought to perform valuable biological services in maintaining a balance of nature in their ecosystem and to fill a scientific need for biological diversity.[12]

Justified as an exercise of the police power to serve the public interest, the Endangered Species Act sought to regulate traffic in these animals at every possible juncture. It became unlawful to take, to buy or sell, or to transport in interstate or foreign commerce any member of an endangered species.[13] The Secretary of the Interior was to designate which species were endangered or "threatened" (likely to become endangered in the future), and to issue rules for their protection. He was given broad land acquisition authority to carry out the legislative policy, and cooperative state and international efforts were to be encouraged.[14] Once more, economic hardship, native subsistence hunting, and scientific research were grounds for making an exception to the mandate.

Like the humane treatment laws, the endangered species laws have as their objective that fewer animals be harmed. But the moral bases for the two policies are quite distinct. The laws protecting species do not rest on a direct moral concern for the individual animals who will benefit from them. They were enacted to advance an aggregate and somewhat abstract public interest in stabilizing the biosphere, where for 'public' one might substitute 'human' without loss of meaning. The difference is not that animals lack the aesthetic, recreational, economic, and scientific interests that were cited in support of legislation. They do after all have an interest in living, and in the first instance that is the interest the laws protect. Only some animal lives, however, are preserved. Protection is conditional on membership in a species that happens to be at risk. The life of an individual animal has value, not when taken alone, but only as a receptacle for the species which it represents for a time.

Is the difference, then, simply between preserving an individual and a species? Not really. "The name of a species," Joel Feinberg has said,

> is not that of some superentity distinct and emergent from the individual animals of which it is composed. Rather, it is simply the name of a collection of entities with certain defining characteristics in common. The species of elephant called *Loxodon africanus*, for example, is not an indidual superelephant with wants and aims, feelings and beliefs. As a species, therefore, it has no interests of its own and is not even the kind of thing that could have a good of its own.[15]

Feinberg concludes that our duty to protect animal species, if owed at all, is owed to other human beings - our descendants perhaps. The animal species is a natural resource, toward which it would not make sense to show humanity.

To grasp the moral point, imagine some individual member of an endangered species that faces a particular threat to its life. Suppose also that it is sterile and that it does nothing to assist other members; in short, it does not make a contribution to the survival of the species. Is there a duty not to take it? By the letter of the law, Yes; for the law takes no cognizance of these special cases. But on the moral thesis which the law embodies, No; for its life is disconnected from the fate of the species. Yet it remains an individual with interests of its own, an individual in need of help. It would be inhumane to take it, without some special reason, and a humane gesture to assist it. The two moral considerations are therefore independent of each other.

Although the endangered species legislation marks a new direction in federal policy protecting animals, it has for that very reason little value as a guide on the matter of animal experimentation. The mouse in the laboratory suffers and perhaps dies as an individual, not as a mere carrier for its species. In general the species put to possibly harmful scientific uses will face little if any threat of extinction; it would be a poor research tool if it did, since the prospects for repeatable experiments would be low. The scientist and one who would add to the protections given animals are not really at odds on the desirability of preserving extant species. The scientist has the right to take specimens where others do not, but the clear presumption behind the exemption in his case is that the taking will be benign - capturing but not killing, done in the interest of knowledge that might help the species to survive.

IV. Animal Experimentation for Public Health and Safety

There is of course nothing in the legislation protecting animals that would prohibit using them in the laboratory when a compelling human need had been shown. Indeed, one might even expect to see a mandate for testing with animals where this was judged necessary to demonstrate that such things as food, drugs, industrial chemicals, and household products are safe for human consumption or use. But that mandate, one might also expect, should contain strict criteria of necessity to express an equally genuine Congressional concern that animal suffering be kept to a minimum. Surprisingly the laws enacted in these areas are comparatively silent on both matters. In at least one respect, however, that silence is a blessing: It preserves consistency in federal policy.

From a moral point of view, what recent public health legislation does say about animals appears to write them off as depreciating assets, their value used up once their function as animate test tubes has been performed. But the language of the laws leaves considerable room for interpretive freedom in the other direction. Let the landmark 1958 Food Additive Amendment to the Federal Food, Drug, and Cosmetic Act serve as a model.[16] It prohibited food processors from using additives that had not been adequately tested for safety. Before then the processor could introduce the additive and market his product until the government had established that it was unsafe. The testing process could take years, and in the meantime a product with an unknown safety record would be widely purchased and consumed. The Amendment required that safety, rather than the contrary, be the fact needing demonstration, and it shifted the burden of proof onto the processor. Its effect was to apply to chemicals in food the standards already in force for new drugs.[17]

The entire statute mentions animals on only three occasions. On the first, the term 'safe' is defined as having reference to the health of man or animal.[18] Since this is merely a definition, it carries no implication that the interests of man and animal are to be equally protected. In light of the testing provisions to come, one might infer that animal health was not to be protected at all, but only to be studied experimentally as evidence of effects on human health. But this, too, would be a mistake. Animal feed was one of the products covered under the law.[19] To be sure, the objective here goes beyond the animal's welfare to insuring that the human food product it may yield is free from contamination; but it remains true that, to secure this result, we would have to protect the health of the animal.

The law lays out a procedure for assessing the safety of an additive under specified conditions of use. But it issues one proviso:

> ...No additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal.[20]

Clearly the testing envisioned **here** is whole animal testing that puts the life or health of the animal subject at risk. And since the clause effectively imposes a burden to be overcome by any proposed additive, it can be read as a _de facto_ mandate for massive experimentation of this kind.

De facto perhaps, but not _de jure_. The law prescribes "appropriate" tests, but does not say what they are. Everything rests on the meaning to be given that term. One sense might be that a test is appropriate if it is 1) practicable and 2) necessary to get reliable results. Whole animal testing may, as a current matter of fact, satisfy both conditions; but should technology make other, equally reliable testing procedures available, it would no longer be appropriate in this sense. Moreover, even if the test is appropriate, it would not follow that it is so in every form. Recalling the Animal Welfare Act, mightn't the appropriate use of anesthetics, etc. be required? Mightn't a limit on the number of subjects used be appropriate? The law itself settles none of these matters.

The same language appears in a later paragraph, where, in making his finding on safety, the administrator is directed to consider, among other relevant factors, those which

> in the opinion of experts qualified by scientific training and experience to evaluate the safety of food additives are generally recognized as appropriate for the use of animal experimentation data.[21]

Here the safety factor rather than the test is said to be appropriate, and the sense appears to be that the administrator is to consider the factors which it would be practicable and necessary to investigate by way of animal experimentation. One could also read into it an unstated assumption that such data are as a matter of due course to be supplied, but the requirement is still intelligible without this. Its meaning is conditional: Such factors are to be considered _if_ there are any. If sufficiently reliable and equally informative alternatives to animal experimentation were to become available, then the answer to the question, Which factors?, would simply be, None. Furthermore, since the statute does not specify which form the animal experiments would have to take - other than, perhaps, that they be as reliable as possible - the law does not compromise any moral or legal imperative to choose the most humane alternative within this set.

The foregoing considerations can be reorganized into a sequence of questions:

1) Does the Food Additive Amendment require whole animal testing as a matter of law? Strictly, no. It requires that the food processor prove that his product is safe, however that may be done.

2) Does the Act require whole animal testing as a matter of fact? It does, _if_ the current state of research is such that this is the only way to establish safety.

3) Does the Act require, as a matter of fact, whole animal testing which inflicts unnecessary suffering? No. To say that the suffering is unnecessary, in the restricted sense, is to say that the experimental result could be obtained without it, and the Act looks only to the result. To say that it

is unnecessary, in the unrestricted sense, is to say that, taking all of its consequences into account, including the value of the additive and its potential dangers, doing the experiment which causes animal suffering has worse consequences than not doing it. The law leaves this judgment to the food processor.

4) Does the Act authorize (i.e, grant a right to conduct) whole animal testing which inflicts unnecessary suffering? No. The Act does not expressly forbid it, but silence is not authorization. If *other* legislation does prohibit it, then that will be the controlling legal consideration.

None of this is meant to deny that administrative findings and regulations with the force of law may stipulate that the processor use certain testing procedures harmful to animals. In this context, however, the administrative rules are best understood as efforts to adapt the legislative guidelines to existing technology. The rules can be and have been changed when the facts change.

In one prominent instance, however, Congress seems to have gone well beyond its usual practice of setting only vaguely specifiable guidelines for testing. The Federal Hazardous Substances Labeling Act of 1960 established a system of nationally uniform warning labels for dangerous household products.[22] They were to serve as a precaution against injury or death that might result either from customary adult use or accidental misuse by children. The information supplied on the label could also assist physicians in giving emergency treatment. A duty to place a correct and conspicuously placed warning on his product fell on the manufacturer. Failure to do so was judged misbranding, a misdemeanor offense. The administrator had the authority to inspect manufacturing facilities and to seize misbranded products after shipment.

The law does not impose any direct duty to test for hazards; it requires correct labeling, and that in turn will make testing necessary. The explicit and implicit references to animal testing are confined to an elaborate classification scheme set up in section 2. A hazardous substance is defined as one which is toxic, corrosive, an irritant, a "strong sensitizer", flammable, etc. to the point of causing personal injury or illness. Each of the subcategories is then defined for purposes of the Act. A toxic substance is one which "has the capacity to produce personal injury or illness through ingestion, inhalation, or absorption through any bodily surface." Although this makes no mention of animal testing, the House Report accompanying the law adds,

> It is the committee's intent that animal experimentation data may, in accordance with appropriate scientific methods, be used as a basis for determining whether a substance is capable of producing injury or illness in man.[23]

This is the language of permission rather than requirement. For other terms, however, the practical implications are stronger. A corrosive substance, for instance, is one "which in contact with living tissue will cause destruction of tissue by chemical action." This does not literally mean that one must test for corrosiveness by placing a substance in contact with living tissue, but the manufacturer who falls under the law might easily infer that that would be the most reliable way to satisfy the legal requirement. And since experiments on living human tissue are presumably ruled out, the best candidates will be animals.

One category, however, precludes even this much interpretive leverage. A substance is "highly toxic", according to section 2,(h), if it

> a) produces death within fourteen days in half or more than half of a group of ten or more laboratory white rats each weighing between two hundred and three hundred grams, at a single dose of fifty milligrams or less per kilogram of body weight, when orally administered...

Two other versions of the LD50 test, one for inhalation and another for body contact, complete the proposed operational definition. As LD50 tests go, these are comparatively mild. Only a small sample is necessary, and one need not keep increasing the dosage until half the animals do in fact die. This said, section 2,(h) remains the clearest case of a legislative mandate to do harmful whole animal testing. And yet even here, Congress displayed reservations. In the immediately succeeding passage, the law adds this qualification:

> If the Secretary finds that available data on human experience with any substance indicate results different from those obtained on animals in the above-named dosages or concentrations, the human data shall take precedence.

Strictly, then, the test is not a definition after all. Toxicity is defined by effects on human beings, and so any data gathered from animals will always be corrigible in principle. The LD50 test is no exception. The law singles it out as the most reliable test then available, but that essentially empirical judgment is not necessarily etched in stone.

The Food Additive Amendment and Hazardous Substances Act sought to protect the consumer from the dangers that new chemicals might present at the marketing stage. Legislation to come stretched back to the point of production, but it added nothing to the role that animal testing was to play in setting safety standards. The Occupational Safety and Health Act of 1970 took note of the increasing number and severity of injuries and illnesses traceable to the workplace, including many chronic diseases apparently arising from continuous exposure to industrial and agricultural chemicals.[24] It authorized the Secretary of Labor to promulgate standards for dealing with toxic materials under working conditions, but beyond this said only that they were to be based on research, experiments, and "such other information as may be appropriate", including the data and experience gained under other health and safety laws.[25] This would no doubt include data and methodological standards for animal experiments, but the law makes no mention of them.

For all its breadth and wealth of procedural detail, the Toxic Substances Control Act of 1976[26] hasn't much more to say about animals. A comprehensive program to protect the public health and environment, it establishes testing requirements and necessary use restrictions for certain chemical substances in the first instance, before they are to be marketed. As in the Food Additive Amendment, the duty to test falls on the producer who would introduce the substance. The law directs the EPA administrator to identify substances for testing and to set standards for developing test data, giving high priority to tests that are efficient and inexpensive. The methodologies "that may be prescribed in such standards include epidemiologic studies, serial or heirarchical tests, in vitro tests, and whole animal tests."[27] Once again the language, at least as addressed to the administrator, is permissive.

Since whole animal testing is expensive and often time-consuming, the law would seem to throw some weight against it; but nothing further is said of this. Nor is there any suggestion that suffering in the animals should be included on the cost side of a cost-benefit analysis. No doubt so little attention is given to such matters because whole animal testing has become such a fixture in the field of public health and safety. Nevertheless, oversight should not be mistaken for a directed verdict in favor of unregulated animal testing. Here as in earlier laws, there is nothing to contradict the presumption, gathered from the humane treatment legislation, against inflicting needless suffering on animal subjects.

Must the judgment which Congress would give if forced to articulate an animal testing policy be left entirely to speculation? It would be, were it not for one instance in which the humane treatment and public safety lines of legislation have managed to intersect.

The Federal Meat Inspection Acts of 1907 and 1967[28] are the major Congressional ventures into regulation of slaughterhouse operations for purposes of public health. They are designed to assure that meat is wholesome, unadulterated, and properly labeled, by conducting regular inspections before slaughter, to separate diseased from healthy livestock, and after slaughter, to detect unsanitary packing conditions, additives injurious to health, or misrepresentation of the meat product.[29] They have tried to strike an optimal balance between the economies of meat production and the welfare of the public; and in this respect, their objective has always been to advance a purely human interest, with nothing mentioned about the welfare of the slaughtered animals.

Yet the Meat Inspection laws function alongside the Humane Slaughter Act. We are not free to interpret the former in such a way as to put them at odds with the humane treatment standards laid down in the latter. The two strands of legislative policy have independent objectives and different sanctions for noncompliance; but barring explicit language that would prevent it, each must be read so as to make it consistent with the other. In 1978, moreover, Congress took a step farther than consistency; it joined the two strands together by adding the following amendment to the Meat Inspection Act:

> For the purpose of preventing the inhumane slaughtering of livestock, the Secretary shall cause to be made, by inspectors appointed for that purpose, an examination and inspection of the method by which cattle, sheep, swine, goats, horses, mules, and other equines are slaughtered and handled in connection with slaughter in the slaughtering establishments inspected under this Act. The Secretary may refuse to provide inspection to a new slaughterin establishment or may cause inspection to be temporarily suspended at a slaughtering establishment if the Secretary finds that any ... have been slaughtered ... by methods not in accordance with the Act of August 27, 1958...[30]

From the standpoint of the Humane Slaughter Act, the Amendment of 1978 strengthens the sanction that attaches to the conduct prohibited there. The slaughterer who uses inhumane methods now faces, not only lost government business, but the loss of his entire business (for a time), since he cannot operate without inspection. From the standpoint of the original Meat Inspection Act, the Amendment adds, as it were, a constraint from the side. It prevents the slaughterer from using certain means to serve the end of human welfare, even if they are just as effective as other methods for promoting that end. He is now required to do something more than whatever will protect the public health. Finally, from the standpoint of legislative policy as a whole, the Amendment makes objectives that were previously pursued independently now joint goals of public policy, but yet in such a way as to preserve the subordination of one to the other. Animal welfare deserves a place in the pantheon of public ends, but not to the point of unseating human welfare when the two come into conflict. The cow cannot be killed painfully, because that is not necessary to advance public health, but it can still be killed.

The Meat Inspection Amendment encourages the thought that a like marriage of legislative ends can be accomplished in the area of animal use for research, testing, and education. At the very least it shows that that possibility cannot be foreclosed.

V. Summary and Conclusions

For many the cacophony of voices in the current debate over animal use in the sciences has been a source of despair. But the dissensus that we seem to see here may be an illusion. The foregoing critical survey of the religious, philosophical, and legislative backgrounds to the controversy gives the wary patient listener some reason to hope that, if the competing voices were muted by reflection, they would begin to coalesce as variations around a single theme. That theme would be the standard of humane treatment, extended to animals as well as men. Given its proper theoretical underpinnings, it emerges as something more than a merely agreeable moral equivalent of muzak.

Much has been made of the historical contrast between Western and Oriental religious views on animals. The biblical and theological texts in the Judaeo-Christian tradition do not give us a principle of unconditional respect for animals. Man alone is accorded inherent value because he alone is created in the image of God, and this gives him a license to use animals for his own purposes. Not, however, to abuse them. Cruelty and callous indifference to the needs of animals find no scriptural support, and even the hardest-line Christian thinkers condemn them. If God is a good shepherd to men, treating them kindly though he is not bound to, men can be as much to the animals in their care. Thus the Christian position amounts to a synthesis of two elements in tension. On the one hand, animals are inferior in worth to man, as the animal body of man is inferior to his soul. On the other hand, they are not so inferior that their own welfare cannot stand in the way of our using them as we please.

Our modern religious and philosophical patterns of thought are branches off the same ancestral trunk. It should not be surprising, then, that the philosophical tradition exhibits the same healthy tension on the subject of animals. The question of comparative worth emerges in the contemporary law conception of ethics as the question of standing. Men have standing as persons - as individuals, that is, who can assume duties and enjoy rights. To join them, animals must at least be capable of possessing rights. But they do not, it seems, have the power of discretion that gives rights their distinctive role in our moral scheme; nor does consistency force us to ascribe rights to animals once we give them to infant and handicapped human beings who also lack discretion. Yet even if animals are not persons, it does not follow that they are mere things, morally indistinguishable from machines. For they are sufficiently like us in one morally relevant respect (their capacity for suffering in basic forms) to generate a moral claim on human agents. It would be inconsistent to hold that, other things being equal, human suffering ought to be relieved, but to deny this of animal suffering.

The rule that suffering ought to be relieved, in men or animals, is the principle of humane treatment. It covers a large and heterogeneous range of situations, among which the most germane, for the debate over animal use, are those in which an agent inflicts suffering on someone else. The humane treatment principle establishes a presumption against doing this, but that presumption can be overcome - always in the case of animals and sometimes even when the victim is a man - by showing that the harm done is necessary to secure some valuable end. Necessity here is not bare utility, although it bears a close relation to the utilitarian principle. The harm must not only be a means to a good end; it must be the only means. Thus necessity always implies a comparison with available alternatives.

Animal use in research, testing, and education creates a conflict of interests between the liberty that men have to use animals for human ends (knowledge, health, safety) and the need that animals have to be free of suffering. There is no reason why either one of these broad interests should always prevail over the other. The fulcrum on which they are balanced is the necessity standard itself; that is, when the suffering inflicted on animals is not necessary to satisfy a desirable human objective, the animal interest will prevail, and when it is unavoidable, the human interest will be controlling.

How should the balance be struck? Congress has enacted major legislation to protect both interests; but while the two strands of public policy have managed to preserve consistency with each other, they have also remained relatively independent. It is still an open question how Congress would choose to combine them, but existing legislation, especially as it incorporates the necessity standard, can provide guidelines for formulating Congressional options. If Congress wished to strengthen the protection given to laboratory animals, it could do so in any or all of the following ways:

i) It could encourage, without mandating, development of alternatives by targeting funds to that purpose, favoring laboratories which use alternatives when grants are distributed, etc.

ii) It could impose a standard of restricted necessity on all research and testing. This would not involve any intrusion into the laboratory by way of evaluating research or testing objectives. The latter can be and often have been set by Congress.

iii) It could place the burden of proof on the laboratory which pleads necessity, as it has done with the manufacturer on the question of safety.

iv) It could impose a standard of unrestricted necessity where the decision is not whether to allow but simply whether to fund a proposal.

The options become increasingly controversial as one moves down the list. And of course as they are omitted or inverted, the human interests served by research, testing, and education will be favored.

All of the options make use of the necessity standard. As that standard is clarified, the prospects for reaching a genuine accommodation of interests on the question of animal use will brighten.

Appendix

To get a more complete picture of a defensible policy on animal use, it may be helpful to examine it from the point of view of an administrator who must put it into practice. Imagine, then, a composite administrator with the legal responsibility to oversee and set standards for research and testing to promote public health and safety, and a legal duty as well to see that these programs are conducted humanely. The law as written gives him enormous leverage in meeting these responsibilities. On the one hand, with the possible exception of the LD50 test (done on a small scale), he is not strictly bound to approve any whole animal testing procedure. More realistically, given that most proposals will still come to him in that form, he is by no means bound to accept them all. He is legally free to investigate and encourage alternatives to or within the domain of animal experimentation. On the other hand, in view of the "hands-off" policy taken in both humane treatment and public health legislation toward research and testing which involves animal suffering, he could ignore that variable if he wished. He will recognize, however, that within these limits some of his options are morally preferable to others. They may be legally preferable, too, in the sense that, as exercises of his rule-making authority, they complete the law in more desirable ways than others.

By what criteria, then, should he reconcile the different and sometimes competing demands of humane treatment and animal welfare? To begin at the theoretical level, he should reject the two extreme positions on animal standing, in part because Congress clearly has and in part because animals cannot at any rate be coherently portrayed as mere machines or as full-blown right-holders.

The decision on standing alone, however, will not get him very far, since it says nothing about how human and animal interests are to be ordered. Legislation has given him the broad outlines of an answer. Human interests have priority in that animals may be used as means to human ends, while human beings may not. Yet if it is not necessary to sacrifice the animal interest in order to satisfy the human objective, the animal interest will win out. The existing law, however, does not spell out a procedure for making these distinctions.

The next step is the critical one. To avoid the charge that any determination beyond this point is essentially arbitrary, the administrator will have to take the standard of necessity seriously. Most people have not. Voicing a widely held view of current bureaucratic practice, Richard Ryder bemoans the British experience with "necessity":

> There is no record of the Home Office refusing a license on the ground that a proposed experiment is pointless, badly designed, repetitive, or wasteful. In other words, the principal loophole in this law, as in all equivalent laws in other countries, is simply that, although painful experiments are technically allowed if they are "necessary", *the necessity itself is rarely, if ever, questioned*.[31]

Ryder subverts his own cause, however, by failing to notice that a pointless and a wasteful experiment may be unnecessary in two quite different ways - the first unrestricted, the second restricted. He then misstates the necessity condition even as he uses it immediately thereafter.

> Only a small proportion of experiments can have more than an infinitesimal chance of being necessary in the sense that they will prolong human life. A slightly larger proportion may be called necessary in as much as they will be published and so may perhaps increase human knowledge, however academic and trivial. For the vast bulk of experiments, however, the only necessities are curiosity, commercial profit, and ambition.

This is to confuse the necessity of an experiment with the value placed on its objective, and so to nourish the misstaken impression that judgments of necessity are at bottom subjective value judgments. The common core of all necessity judgments is that there is some state of affairs which can only be realized in one way. Thus whatever the value of that state of affairs, the necessity judgment will hold only if there is no other means of reaching it. This question is properly a matter of fact. It may be, then, that one can only establish necessity by defending a value judgment, but a failure of necessity can be shown on the facts alone.

Perhaps without realizing it, administrators make these negative necessity judgments all the time. Comparative cost-benefit analyses are necessity judgments. If two experiments will yield the same results, but one is more expensive, the additional expense is unnecessary. If two others have the same result, but one is less efficient, the extra resources it uses up are unnecessary. Why not then say that, by the same token, if one experiment inflicts more animal suffering than another, but both get the same result with the same reliability, the surplus of suffering is unnecessary?

These are all restricted necessity judgments, made from a perspective internal to the procedure itself. As long as they are supported by competent professionals and administrators who rely on their expertise, such judgments pose no threat whatever to research objectives or methodology. They could not do so, since these two considerations are their point of departure.

It is true that a judgment about the unnecessary expense of an experiment and a judgment about the unnecessary suffering it involves will likely have different policy implications. The first typically supports an economic decision, backed by a legislative mandate to husband public resources; the second will underlie a moral decision, backed by a legislative requirement to give animals humane treatment. But in neither case will any research interest be compromised. The research objective will be met by the alternative with an equally acceptable methodology but with less economic or moral cost.

Accordingly, if any proposal would inflict unnecessary suffering on animals, in the restricted sense, relative to some alternative like it in all but this respect, the administrator will have a compelling reason in morals and public policy to reject it. There may not be many such cases; but the principle is worth stating, if only because so many have denied that research and testing are subject to such judgments. But if they allow them in the economic case, they will have no good reason to deny them in the moral case.

The far more common case within the domain of animal experimentation, especially where toxicity testing is being done, will be one in which an argument exists for using more animal subjects or withholding anesthetics, viz., that this will assure more reliable results. Such reasoning might support a request to fund an LD50 test that would use sixty rather than ten rats. Is the additional suffering in this case unnecessary? Would the administrator, no longer able to choose between the two alternatives on restricted grounds, be obliged to fund the larger experiment?

Clearly there are economic limits to arguments that reduce to "The more, the better." Nor is this point as trivial as it may appear; for it means that some judgments of unrestricted necessity are permissible even in the research and testing context. Moreover, they are particularly applicable where, as in the case of toxicity testing, the experimenter borrows his research objective from public policy. The goal here is not pure knowledge but knowledge for a particular purpose, to protect the public health. Now suppose that the administrator were to choose between the ten- and sixty-rat proposals on economic grounds. He might reason that, though the sixty-rat option will buy more reliability, he does not need the extra reliability to protect the public health. He can learn enough from the ten-rat test to estimate the health risk, or at least judge whether further testing is needed; and as long as doubts are resolved on the side of caution, the money would be better spent on small-scale testing of other substances, or on purchasing more reliability where that would make a greater difference - say, in basic cancer research.

The experimenter who fails to obtain funding for the larger project may grumble at his loss and even quarrel with the particulars of the administrator's reasoning; but his complaint will not extend so far as to imply that the kind of reasoning which the administrator used invaded the prerogative of the scientific community. It would have, perhaps, if it were a case of pure research, but not here, where the very basis of the experiment lies in the service it performs for public policy.

The economic element is again but one of many that make up public policy. Constraints on the treatment of animals constitute another. If so, there should be no bar in principle to a union of humane treatment and public health desiderata in decisions on funding of research and experimentation. The wise administrator will no doubt leave such a question for Congress to judge, as it did in the case of meat inspection. But Congress might begin by reminding all participants in the debate that there are no sacred cows.

NOTES

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CHAPTER 3

SCOPE OF "ALTERNATIVES": OVERVIEW OF THE STATE OF THE ART.

by

Roland M. Nardone and Lucille A. Ouellette

A. CONTINUED USE OF ANIMALS WITH MODIFICATION

1. Reduction in Numbers:

The data presented in Chapter 2 reveal that very large numbers of animals are used for diverse purposes ranging from basic scientific research and education intended to better understand how living organism and its parts function, to medical education and research geared to the understanding of disease processes and how to prevent or cure diseases, to toxicity testing which is geared to prevent the unintended exposure of humans and other organisms to toxic levels of harmful substances. These potentially harmful substances may be environmental chemicals, such as auto exhausts, pesticides, and flame retardants on clothing, to products which are ingested or otherwise taken internally (food, medicinals). Furthermore, the trend has been to increase the commitment of funds to life science research and to be more demanding in regard to the testing of chemicals before they are released into the environment. Much of the impetus for the latter stems from enactment of the Toxic Substances Control Act (TSCA) in 1976.

In view of the many diverse segments of the scientific community involved in research, testing, and education which may utilize animals, it is hard to generate reliable figures regarding actual use for all purposes.

Rowan (1984) reported that about 5.7 million animals are used for education programs, about 28.5 million for research programs, and about 14.3 million for toxicology programs, which included toxicity testing. These figures translate as follows: 8% of all laboratory animal use is for teaching, 40% is for research and 20% is for toxicology.

In order to understand why testing requires so many animals, let us survey the conduct of a representative toxicological evaluation for carcinogenesis using rodents.

Note that every toxicology-based study has at its core the dose-effect or dose-response study. Unless a correlation can be demonstrated between dose and magnitude of toxic response, good proof would be lacking that the effect observed is indeed due to toxicity (Klaassen and Doull, 1980). Hence, in this as well as other toxicity studies, several doses of the test chemical are used.

The level of complexity of toxicity testing experiments depends on the outcome of earlier experiments with the test chemical and related chemicals, the target tissues which are likely to be affected, how the chemical will be metabolized and distributed within the body, and the nature of the lesions to be caused.

For example, in designing a cancer evaluation study, the toxicologist takes into consideration that the time of appearance as well as the number of cancers induced will be positively correlated with the dose. However, it takes about 1/8 of the animal lifespan for the first tumors to appear. Also, some animals may die from factors unrelated to the test chemical including spontaneous tumor formation. Furthermore, the level of certitude and the limits of detection of the evaluation will be additional considerations which affect the number of animals to be used.

In order to evaluate alternatives to animal use and ways to reduce the number of animals used, it is important to recognize that there are many branches of the science of toxicology, some of which have a common trunk but different objectives. These different objectives often require different methodologies. Klaassen and Doull (1980) classify the professional activities of toxicologists into three major categories: descriptive, mechanistic, and regulatory. Toxicologists engaged in any one of these primary categories may focus on one or several different aspects such as carcinogenesis, mutagenesis, and systemic toxicity (including the many subsets such as hepatotoxicity, renal toxicity, ocular toxicity). Hence, in addition to the different objectives, the methodologies will reflect the primary category. For example, Gralla (1980) lists the following as primary objectives for safety evaluation.

1. Identify the primary target organ(s).
2. Establish the reversibility of the toxic lesion(s).
3. Determine the most sensitive diagnostic method(s) for detecting the toxicity.
4. Indicate toxic mechanisms, if possible.

Hence, any alternative to the animal testing model will have to be able to satisfy objectives (1), (2), and (4).

The following information, including Table 1, is excerpted from the National Cancer Institute Guidelines for Carcinogen Bioassay in Small Rodents (1976) and illustrates the recommended procedure for carcinogenesis testing. The Office of Science and Technology policy has published similar guidelines in The Federal Register 49, May 22, 1984.

ACUTE TOXICITY STUDY Need: An acute toxicity study may be necessary when data are unavailable as to the toxicity of the test agent. Its purpose is to determine the lethality of the test agent as well as to gain other information on its acute toxicity. The data collected should be used as a guideline to determine the dose range to be used in subsequent studies designed to predict the maximum tolerated dose (MTD).

Experimental Groups: To determine the lethality of the test agent, at least five animals (no older than young adults) of each sex and strain to be studied in the chronic phase should be used at each of the dose levels to be tested.

Dose Levels and Observation Period: The lethality of the test agent can be estimated by use of a series of three or more dose levels which increase in a geometric progression; e.g., by a factor of two. For the purpose of this study, a precise lethal dose (LD) value need not be determined. 14-day observation period.

REPEATED-DOSE STUDY Need: The 14-day repeated-dose study should be done when the available toxicologic data are insufficient to determine the dose levels to be used in the subchronic study.

Experimental Groups: Five animals (no older than young adults) of each sex and strain to be studied in the chronic phase should be used at each of the dose levels to be tested. A similar number of comparable animals should be used as controls.

Dose Levels and Observation Period: Five dose levels should be initially tested. The upper level should be the one that is estimated to produce no more than 10% lethality following administration of a single acute dose of the test agent. The remaining levels should be fractions thereof; e.g., one-half, one-fourth, one-eighth, etc. If these fail to provide the information needed to select the dose range to be used in the subchronic phase, the study should be extended using

TABLE 1. - CARCINOGEN BIOASSAY SUMMARY TABLE

STUDY	PURPOSE	NUMBER OF DOSE LEVELS	NUMBER OF ANIMALS PER DOSE	OBSERVATION PERIOD
Acute Toxicity	To determine overall toxicity of test agent	3 or more	5 of each sex and for each of 2 strains	14 days
Repeated-Dose Study	To determine doses to be used in sub-chronic study	5	5 of each sex and for each of 2 strains	14 days
Sub-chronic Study	To predict long-term toxic effects and maximum tolerated dose	5	10 of each sex and for each of 2 strains	90 days
Chronic Study	To determine carcinogenicity of test agent	2 or more	50 animals of each sex and for each of 2 strains	3-18 months

the initial results to determine whether higher or lower doses should be further tested. 14-day observation period.

SUBCHRONIC STUDY Need: The purpose of the subchronic study is to predict the toxic effects which may occur in the animals during the chronic administration of the test agent. Based on the subchronic observations, a prediction is made of the maximum tolerated dose (MTD) that can be given to the animals in the chronic study without producing unwanted side effects.

Experimental Groups: The experimental groups composing the subchronic study should include the test and appropriate control animals of each sex and strain to be used in the chronic study. Each experimental group should contain 10 animals of one sex and strain at a single dose level. The age of the animals should be the same as those to be used to start the chronic study.

Dose Levels and Observation Period: Five dose levels should be tested -- the highest being the one that does not produce clinical signs of toxicity, pathologic lesions, or mortality in short-term acute or repeated-dose studies. The remaining dose levels tested should be fractions thereof; e.g., one-half, one-fourth, one-eighth, etc. If these fail to bracket the range in which the MTD can be predicted, the study should be extended and the information from the initial attempt used as a guide in setting the dose levels for the next series to be tested. Observation period 90 days.

MTD Determination: A MAXIMUM TOLERATED DOSE (MTD) should be selected for each sex of each strain to be used in the chronic study. The MTD is defined as the highest dose of the test agent given during the chronic study that can be predicted not to alter the animals' normal longevity from effects other than carcinogenicity. The MTD is estimated after a review of the subchronic data. Since these data may not always be easily interpretable, a degree of judgment is often necessary in estimating the MTD. The MTD should be the highest dose that causes no more than a 10% weight decrement, as compared to the appropriate control groups; and does not produce mortality, clinical signs of toxicity, or pathologic lesions (other than those that may be related to a neoplastic response) that would be predicted to shorten the animal's natural life span. Other measurements also may be used to aid in predicting the MTD.

CHRONIC STUDY Need: The purpose of this study is to determine the carcinogenicity of the test agent in both sexes of two species and is designed to cover the greater part of the animals' life spans.

Experimental Groups

Animals Groups - Each experimental group is composed of animals of the same sex and strain receiving an identical treatment. The usual experimental groups used in the chronic study are shown below.

Test Groups - Each test group is composed of animals exposed to an identical dose level of the test agent.

Control Groups - One or more different control groups may be necessary to evaluate the carcinogenicity of the test agent. The most common are:

Vehicle control groups - Animals in these groups are treated with only the vehicle in which the test agent is dissolved or dispersed.

Untreated control groups - Animals in these groups should be identical in every respect to those in the other experimental groups except that they receive no specific treatment.

Although the above controls may be adequate, the appropriate matched control groups necessary to evaluate a test agent's carcinogenicity also may be those receiving another material or exposed to a given procedure used in its administration. Except for the specific treatment, all experimental groups should be identical in every respect, including source and conditions of maintenance.

Animal Age/Group Size. At the start of the chronic study, animals should be no older than 6 weeks of age and, if possible, weanlings. Each test and appropriate control group should contain 50 animals. When several test agents are placed on study at the same time, common untreated and, where applicable, other control groups may be used. The size of such control groups should be increased by a factor equal to the square root of the number of test agents to which they are common. Thus a control group common to 4 test agents would contain 100 animals ($100 = 50 \times \sqrt{4}$). Common control groups should be located in the same animal room as the test groups are housed.

Dose Levels and Observation Period. At least two dose levels of the test agent should be studied in each sex of each strain. The highest dose should be the MTD, as predicted from the subchronic data, and the remaining level(s) a fraction thereof. In instances in which the test agent does not cause signs of toxicity at levels up to 5% (50,000 ppm) in the diet, it is recommended that this concentration not be exceeded except under special circumstances; e.g., when the test agent is used as a major human dietary constituent. If clinical signs of toxic effects appear during the course of the chronic study, appropriate action must be taken to ensure maximum animal survival. The type of action will depend upon the objective of the study and may include lowering the dose, discontinuing treatment, or changing the dose regimen. Although the doses may need to be adjusted downward during the course of the study, they should never be raised. Frequent and competent animal surveillance must be constantly stressed. Observation period - usually 3-6 months; sometimes 18 months.

Of course, other kinds of testing may not involve chronic exposure or toxic effects which occur only rarely. Such studies would not require as many animals and the observation period would be reduced..

The concern about the large numbers of laboratory animals used, has spawned efforts to reevaluate the basis of animal use in three major areas - testing, research and education - in an attempt to reduce the number of animals employed. The ensuing section of this document addresses the major issues and approaches for each area and concludes with a consideration of advantages and disadvantages.

Use of Animals for Testing. Toxicity tests fall into two major categories: screening tests and risk assessment test. Screening tests are intended for the rapid, inexpensive evaluation of the potential toxicity of large numbers of chemicals. The tests tend to be short-term (acute) toxicity tests which include high doses in order to have the toxicity become evident in a short period of time (see Acute Toxicity Study section of NCI Guidelines, page 3). As often as possible, the end-points selected for observation are those which can be evaluated by a trained laboratory technician but which do not usually require unusually specialized skills, like those of a pathologist. The animals most often used are mice and rats, although certain compounds and tests (e.g., eye irritancy) may entail the use of other species.

For most screening tests, a predetermined range of doses and a single route of exposure are used. Testing of other doses and routes are not automatically undertaken. Often, the test may enable the tester to label the substance toxic or non-toxic or at times assign some quantitative or semi-quantitative designation of the degree of toxicity. The array of positive and negative controls is small or non-existent.

Risk assessment testing is intended to inform the toxicologist of the potential risk of a particular constellation of exposure conditions which include the sub-populations at risk, (children, pregnant women, males etc.), the dosages to be encountered including concentration and exposure duration, route of intake, and environmental factors. Because one extrapolates from animal toxicology directly to human risk assessment, greater precision and certitude are required than for screening tests. In order to achieve such certitude and precision large numbers of animals are used. The number used is increased further because more parameters relating to exposure conditions are involved. Because it is necessary to mimic the probable circumstances of human exposure, such studies may involve low doses administered over a long period of time. Under those circumstances, large populations may have to be exposed before a statistically significant number of animals show some toxicity. Also, many of the end-points may require evaluation by highly trained and experienced personnel.

Because screening tests are minimal tests which have been designed to save money and time, the number of ways animal use may be reduced is limited. Among the possibilities are 1) accept a reduced level of certitude stemming from the use of fewer animals; 2) combine selected tests which allow the same animal to be used for two or more concurrent tests or sequential tests which probably do not interfere with one another (e.g., an eye irritancey test with a muscle strength test); 3) run two or more tests concurrently and have the same control population serve each test group.

The role of risk assessment makes the first two modifications in test design unacceptable for risk assessment testing studies. The advantages of concurrent tests with a common control is discussed below in the context of protocol changes after research use of animals is considered.

Use of Animals for research. An analogy exists between the needs for screening and risk assessment testing on the one hand and pilot and definitive biomedical research experiments on the other. Biomedical research often entails preliminary, "quick and dirty" pilot experiments which may then be redesigned and expanded for definitive studies. Hence, much of what was said before about ways to reduce animal use for testing is applicable to biomedical research.

Pilot research experiments should be designed to use as few animals as possible without nullifying the intent of the research. A sacrifice of precision for savings is the basic trade-off. The definitive research experiments require precision. Hence, reduction in animal use in a well-designed experiment is not a likely possibility.

However, researchers can usually recount many personal and other experiences where accumulated experimental data were invalid or useless because of a poorly designed or executed experiment - situations where the experiment did not test the hypothesis in an unequivocal fashion or where the experimenter was not familiar with an important bit of background information, which would have led to the abandonment of a planned experiment.

Another area which merits discussion concerns the repetition of published research. Certainly, if experiments were not repeated, animal use would be reduced. The issue then, is how important is the repetition of such experiments. The occasional reports of fraud - indeed the nature of science as a self-correcting enterprise which embodies a certain level of skepticism, will insure that many experiments will be repeated. Most scientists will limit the repetition of experiments to the following situations: a) when repetition of a facet of the experiment is needed to perfect a technique or to gain assurance that the experiment is being properly performed, b) when there is reason to be suspicious of the reported outcome, and c) when the importance of the research report is so great that it compels substantiation. There is so much new and interesting work to be done that only these circumstances will motivate a scientist to expend time and resources in repeating an experiment.

Use of Animals for Education. Approximately 9% of laboratory animal use is for teaching. Such use may involve the following: specimens used primarily for the teaching of anatomy and dissection skills; live, whole animals used for behavioral observations and other non-intrusive studies (e.g. growth); live, whole animals used for intrusive studies such as those that require blood collection; live, anesthetized animals used for intrusive physiological studies such as modification of circulatory pathways and evaluation of damaged organs and tissues; animals used as sources of freshly isolated body parts - removed after sacrificing the animal by one of several methods such as cervical dislocation, asphyxia, or drug overdose; live anesthetized animals used for the development of surgical skills such as those required for certain physiological studies and for medical procedures (e.g., cancer surgery and gun shot wound correction).

Those who seek a reduction in animal use suggest that the reduction could be achieved by increasing the size of the laboratory teams, by using the same animal for more than one purpose, by eliminating those laboratory exercises which involve the repetition of procedures which were experienced elsewhere, by relying more heavily on films, video tapes, and other visual aids, by substituting live demonstrations for hands-on experience to those for whose training it is essential.

Reduction in Numbers Stemming from Protocol Changes. It has been suggested that many more animals are used for toxicology than are needed (Rowan, 1984) that changes in experimental design could result in a substantial reduction in the numbers of animals used. In order to evaluate this premise it should be borne in mind that there are many kinds of toxicology studies and each kind - indeed, each specific experiment - may have its own unique requirements. Nevertheless, there are some basic principles which guide the scientist in the design of the experiment and the determination of the number of animals to be used. The following summarizes some of these principles as applied to toxicity testing and research.

1. What level of certitude is required (desired)? While scientists desire the highest level of certitude possible they recognize that science deals with an imperfect system of sliding scales of probability and absolute certitude is impossible. Hence, scientific custom says that an "effect is real" or a "scientific fact exists as a fact" if statistical analysis indicates that there is a 5% chance or less that what is being observed is due to chance, i.e., not related by the presumed cause and effect. Hence, they seek confidence estimates of 0.95 (95%) or higher when experiments are performed. Can lower confidence levels be acceptable? It depends. Obviously, if one was studying the toxicity of a drug intended for human use, the answer would be "No".

On the other hand, if the study was a screening type of study to be followed by other studies, or if definitive conclusions were not to be drawn, one could use to advantage some information from a study with a reduced level of certitude. However, the cumulative experience of science emphasizes that this could lead to faulty conclusions which, in turn, could mislead others and result in wasteful experiments which could result in the wasteful use of animals.

Along with certitude is the consideration of needed precision. For example, the LD_{50} acute toxicity test is designed to determine with statistical accuracy that dose which kills 50% of the treated animals. In order to obtain such precision one must minimize the standard deviation. Thus, the number of animals used at each dose level is kept high. On the other hand, if this level of precision is not required - if a qualitative estimation of toxicity will suffice - the LD_{50} test could be replaced by the determination of the Approximate Lethal Dose (ALD) which has been reported to be adequate for expression of the degree of toxicity of a chemical (Deichmann and Mergard, 1948; Rowan, 1984).

2. How much of an effect do you need or desire to detect? A study which is intended to detect a minute effect or rare event will use far more animals than one which is intended to detect a readily observable or common effect. In the ED_{01} study conducted by the National Center for Toxicological Research, the experiment was designed to find that dose of a known carcinogen, acetylaminofluorine, which would cause one cancer in 100 mice. It required 24,000 mice. To find the dose which would cause one cancer in 1,000 mice would require the use of 80,000 mice (Cairns, 1979).

3. Is the manifestation of the toxic effects apt to be influenced by species or strain differences, by sex, by age, by biorhythms or natural environmental variables such as temperature and population density? If the answer is "No" then a single population of sexually mature males would probably suffice unless proved otherwise. However, we know from many toxicity studies that the list of extrinsic and intrinsic factors which can affect the toxic response is very large, and a series of experiments controlling for each of these factors is often warranted.

A review of the information presented above suggests the following.

1. High levels of certitude usually are required.

2. The scientific problem that is posed often dictates the size of the animal population to be used. Hence, experiments which seek

minor changes or rare events as a result of exposure to very low concentrations of a toxin will use large numbers of animals. When such experiments are warranted, as they often are, the use of large numbers of animals is also warranted, from a scientific point of view. When such experiments are not warranted, then there will be the unnecessary use of animals.

3. The ideal experiment, from the point of view of numbers of animals used, is one which uses the smallest number of animals in l dose-response and other studies while providing the desired level of certitude. Hence, procedures which will assist in predicting the toxic range of concentrations to be used and which will provide preliminary information regarding the magnitude of the effect to be observed will be of value in scaling down an experiment. Such information can be obtained by range - finding pilot experiments which employ few animals and a broad range of doses. However, a precise extrapolation from a pilot experiment to a larger experiment is sometimes difficult and could lead to significant set-backs or failures. For example, a rat carcinogenesis study usually is a two-year (rat lifetime) study. Imagine the waste of time and resources which would occur if, in an attempt to use as few animals as possible, one miscalculated only to discover at the end that the number of animals used should have been larger. In order to protect against this eventuality most investigators set-up experiments with a considerable margin for error in extrapolation.

This should not be implied to mean that there is no room for reduction in animal use by improved statistics and extrapolations from pilot experiments. For example, a two-year pilot study on carcinogenesis is not the basis of planning a two-year major experiment. Instead, a short-term pilot experiment employing few organisms and high doses of the test chemical provides the basis of the design of the major experiment which will use low doses and long exposure time. Basic research in this area is needed as are retrospective analyses of the successes and failures associated with such extrapolations. Note, however, the widespread criticism of the rat-saccharin experiments which demonstrated saccharin to be carcinogenic only when high doses of the sweetener were used, doses which had questionable bearing on human risk.

The number of animals used for experiments is reduced when several chemicals are tested simultaneously. It is possible to have one control group serve as the control for several experimental groups. The size of the control groups is determined by the formula $A \times n$ where A is the size of each group and n is the number of simultaneous experimental groups (Weisburger and Williams, 1980). Hence as the number of treated experimental animals increases due to more groups being tested simultaneously, the increase in size of the control (untreated) animal group increases, but to a lesser extent. Thus, one control group for four groups of 10 experimental animals should number 20 ($10 \times \sqrt{4} = 20$) and a total of 60 animals (20 controls and 40 experimentals) would be used. One control group and sixteen experimental groups, used simultaneously, would require a total of 200 animals (40 controls and 160 experimentals). Thus, a four-fold increase in the number of experiments (from 4 to 16) results in a 3.3-fold increase in the number of animals used.

Reaping such benefits is easier said than done. Coordination of several experiments is often difficult and requires depth of staff and an abundance of reserve space in the animal quarters.

The importance of experimental design and selection of a statistical approach on the number of animals required for statistically significant experiments is illustrated by Table 2. The table compares the numbers of animals which are required for Control and Test Animal groups when a single dose of a drug is tested for carcino-genesis. Three situations are presented and compared using a One-Sided Fisher's Exact Test and a Two-Sided Fisher's Exact Test. The One-Sided Test uses different numbers of Control and Test animals (500 and 50, respectively) while the Two-Sided Test uses identical numbers of Control and Test animals (500 each). The three situations reflect differences in frequency of spontaneous tumors in the Control group (0%, 20%, and 40%). For each situation, and for each statistical test, different percentages of test animals must develop tumors before the drug can be designated as carcinogenic. As can be seen from the table, fewer animals need to develop tumors but many more animals are required for the Two-Sided Test.

Because scientific experiments do not provide absolute certitude, experimenters may increase their confidence estimate by increasing the number of trials, i.e., by repeating experiments. The value of repeating experiments is inversely proportional to the confidence (probability) estimate of a single experiment. However, several repeat experiments which consistently produce a p value of less than .95, ordinarily are not to be preferred to a single experiment with a .95

Table 2. -

Smallest Statistically Significant Percentage of Animals with Tumors on a Drug Dose (Pd) Compared to Animals with Varying Percentages of Tumors (Pc).

Based on Fisher's One-Sided Exact Test at P 0.05

% Controls with Tumors	Number of Animals		% Test Group with Tumors
	Controls	Test Group	
0	500	50	4
20	500	50	32
40	500	50	54

Based on Fisher's Two-Sided Exact Test at P 0.05

% Controls with Tumors	Number of Animals		% Test Group with Tumors
	Controls	Test Group	
0	500	500	4
20	500	500	26
40	500	500	47

or higher p value. Furthermore, repetition of an experiment with a high p value provides little additional improvement in the confidence estimate. From this it follows that animal use could be modified by weighing the trade-offs in money, numbers of animals used, and level of certitude required. At times, three trials with 20 animals each may provide an acceptable level of certitude and substitute for a large scale experiment which uses a hundred animals.

The coordination of a series of studies which employ the same animals for several studies performed in sequence is fraught with difficulties in logistics and suitability for good science. Nevertheless, it is possible to envisage experimental situations whereby control and lightly affected animals from one toxicity study may be useful for very preliminary investigations in a subsequent study - perhaps as a preliminary to a pilot study.

The disadvantages to this approach stem, in great measure, from the uncertainties and unknown linkages in the toxic effects which may exist. The net effect of serial testing is most often going to result in bad science, less-than-ideal utilization of personnel and other resources, and the generation of data which is suspect.

In addition to possibly linked toxic effects, the very act of handling the animals again and in a different way may affect the response of the animal. Certainly, studies in behavior toxicology and other studies which involve bio-rhythms would be most readily affected.

Sharing of controls, i.e., one group of control animals serving as a control for more than one test or research group, is not inherently bad. Its principal limitation is logistic. The logistics difficulties are least in those commercial laboratories where many compounds are being tested continuously. The logistics for a biomedical researcher would be formidable.

Advantages and Disadvantages of Reduced Animal Use in Research and Testing. The advantages stemming from a reduction in the number of laboratory animals used for testing and research extends beyond humane and economic considerations. Reduction leads to savings of time and money which can then be redirected toward the testing of additional chemicals, the more thorough testing of others, and the exploration of other research topics.

The disadvantages are several. The sacrifice of precision may lead to the failure to detect a significant phenomenon. Hence, it would be prudent to sacrifice precision only if it did not interfere with the opportunity to detect; however, most often these are linked.

In toxicity testing "false positives" and "false negatives" occur. That is, some chemicals which are harmless may test out as harmful (false positives) while tests of other chemicals may show them to be safe but in reality they are harmful (false negatives).

The risk to society is greater from false negatives (chemicals which are unsafe but which are treated as if they were safe) than for false positives. Hence, when tests are developed and validated, the frequency of false positives and negatives are scored and that information is used in evaluating the outcome of screening and risk assessment tests. As the precision of a test is reduced, so is the ability to get a good assessment of the frequency of false negatives and false positives.

Advantages and Disadvantages of Reduced Animal Use for Teaching. Visual aids, work with preserved speciments, and demonstrations do not reproduce the experience of working with live material. For the training of physicians, biologists and toxicologists such a limitation may be very significant. For liberal arts students the limitation may be less significant.

The first-hand experience in education serves several roles. It shows things as they really are. The fragility of blood vessels, the elasticity of muscle, the delicate nature of nerves cannot be appreciated from preserved specimens or visual aids. The hands-on experience of working with live animals serves to desensitize some future scientists regarding work with live animals. Such partial desensitization, if it does not result in indifference, helps a scientist get over the apprehension and jitters associated with such work and concentrate on the task at hand. Such experience also assists people in evaluating their suitability for a biomedical career.

The principal advantages from the implementation of measures to reduce animal use are in cost benefits, time benefits, and a reinforcement of the view that animals should not be used except for very serious reasons.

2. Reduction in Degree of Insult.

The humane treatment of animals has long been espoused by biomedical researchers. Yet failure to provide such humane treatment in all instances has prompted the introducton of legislation and official pronouncements which specify what is considered to be humane treatment and to specify the circumstances under which painful experiments can be performed (Pratt, 1980). It is the conduct of necessary painful experiments which will be considered here as well as the approaches which are feasible for their replacement or modification.

Custom and law approves of the conduct of painful experiments on animals when specific circumstances apply. These include the importance of the experiment and the imperative that the animals are not anesthetized or given analgesics pre-or post-experimentation. The reporting of such experiments on some animal species (mice and rats are excluded) is mandated by the Animal Welfare Act administered by USDA.

Importance is a relative matter and it may be impossible at times to get unanimity of opinion regarding how important a given experiment may be. Are all experiments which bear on life-threatening diseases important? If not all experiments, what about all diseases including those which are extremely rare? Are all experiments which relate to cosmetic development and use to be considered trivial? Nevertheless, a consensus usually develops among those engaged in research regarding the relative importance of the majority of biomedical experiments which employ animals.

Pratt (1980) enumerates the following as reasons given the USDA for not using anesthetics during some experiments.

1. The anesthetics would mask the reaction being noted.
2. The mice were used as objects for mouse-killing rats in studies of anti-depressant agents.
3. The behavior of the animals would have been modified by anesthetics.
4. Anti-inflammatory reactions would be interfered with.
5. In order to mimic a human stressed situation which leads to gastrointestinal ulceration, the animals were not anesthetized.

These reasons are typical of those offered by biomedical researchers. The anesthetic interferes with the detection of a phenomenon, the evaluation of a phenomenon, or the development of the phenomenon of interest.

Insult During Teaching. One would be hard-pressed to describe a scenario in a teaching situation which would require the use of unsedated animals for painful experiments. Teaching situations afford opportunities to reduce the level of insult, to modify how the exercise is performed, and to terminate the animal's life as soon as the desired principle has been demonstrated.

Pain, physical and psychological, may be experienced by laboratory animals. Hence, both should be recognized in an evaluation of the problem.

Psychological pain may result from separation of a social animal from its relatives, from the learning it has developed which may enable the animal to anticipate a painful experience, and from the treatment that the animal has received in captivity. Obviously, not all species are equally influenced by such factors.

Insult During Testing and Research. Experimental testing and research procedures vary in regard to the permissable level of sedation, from none to heavy, and in the levels of inflicted psychological and physical trauma. The trauma may be momentary or persistent throughout the experimental period. Hence, an evaluation of the problems which confront the researcher is complicated.

Experimenters will not use unanesthetized animals for painful experiments unless there are specific needs for experiments under such conditions..

Research experiments in Sweden are classified according to levels of pain into the following categories: 1. Experiments which, with or without operation, are expected to cause only negligible pain or agony. 2. Experiments carried out on anesthetized animals, which do not wake up again. 3. Experiments with painful stimulation of unanesthetized animals, which causes the animal short-lasting, light pain. Operations are carried out under anesthesia or some other pain-killer, but the animal will wake up again or experience the cessation of the action of the painkiller. The animal can then have transient postoperative nausea, more or less severe. 4. Experiments on unanesthetized animals of whom some can be expected to become seriously ill or be caused significant pain or significant agony. 5. Experiments on unanesthetized and curarised (or equivalent) animals.

Ross (1978) has reported that of 218 experiments, 83 were comparable to class 1, 71 were class 2, and 39 were class 3 experiments. Twenty-two experiments involved heavy pain.

Advantages and Disadvantages of Reduction in Degree of Insult During Testing and Research. Psychological and physical pain may be reduced by measures which allow for the animals to become accustomed to handling and by an individualized, deliberate approach. The primary benefits to be gained are as follows. Humane treatment will be afforded. The animals will be more normal. Such animal care and handling may require more careful selection of lab personnel and more extensive training. It may also lead to reduced productivity.

There are no advantages other than scientific ones associated with the occasional use of non-sedated or unanesthetized animals in painful experiments. The benefits are many and include more uniformity in the execution of a procedure, easier animal care, and avoidance of the animal mutilating the damaged area.

Advantages and Disadvantages of Reduced Insult in Teaching Situations. The avoidance of painful experiments instills in students a reverence for life which transcends the laboratory experience. It serves to insure that future biomedical scientists do not become callous. Also, the advantages cited earlier - normal performance and ease of handling - are important considerations, especially for the novice.

3. Use of Lower Vertebrates.

The classes making up the lower vertebrates include the fishes, amphibians and reptiles. These animals are all cold-blooded meaning that their body temperature is unregulated and therefore the same as ambient temperature. Furthermore, these animals have much smaller brains than the higher vertebrates and the olfactory lobes are the more predominant portion with the cerebrum being underdeveloped. Because the cerebrum is the center for much sensory activity, it has been suggested that these animals could serve as good alternatives because they might be less susceptible to pain. This hypothesis has yet to be tested and clearly defined.

Use of Lower Vertebrates in Testing and Research: The fact that the lower vertebrates are so far removed from man and have basic physiological differences, makes them less useful sources of alternatives for testing purposes. On the other hand, they have been found useful in research. Expecially research related to toxins that accumulate in an ecosystem by increasing in concentration as one animal eats another. In many cases the toxin is not toxic to certain animals and consequently they survive to be ingested by other animals. This causes the toxin to be concentrated in the tissues of unaffected animals and be more lethal to animals higher in the ecosystem.

The fishes are also useful barometers of toxic substances that accumulate in water from industrial waste. Dose responses could be performed in the laboratory on the fish that are found in the waste waterway and certain maximum levels of toxic waste could be defined before a plant goes into actual production.

The primary use of lower vertebrates in research is restricted to the amphibians and fishes. Several cell lines have been isolated from fish and established in cell culture. These cell lines can be used for in vitro studies of fresh water and salt water toxins. More recently, Philip Harris Biological has developed a Xenopus (Frog) Kidney Tissue Culture Kit which provides an introduction to cell culture and its potential uses to college students. The Fund for the Replacement of Animals in Medical Experiments (FRAME) sponsored several kits for interested schools (FRAME, Technical News, 1979). In general, however, use of these animals as alternatives is confined to education.

Use of Lower Vertebrates in Education: Organisms from these classes have been used in education, from grade school level on up through universities, for decades. In fact, Rowan reports that in 1969, approximately 9 million wild-caught frogs were shipped by U.S. suppliers to educational institutions. Of these, just under 50% were preserved preparations, the remainder being alive (Rowan, 1984). Prior to that time, the emphasis had been on the study of biology, with emphasis on preserved animals.

Frogs are excellent specimens for anatomy and physiology laboratory exercises and can thus be used in the preserved state. However, to demonstrate the beating of the heart and circulation, live animals have been employed. The suggestion has been made that perhaps audio-visual tapes could be used to accomplish the same objective. In this way only a few animals would need to be sacrificed and several duplicate tapes could be produced and sold as instructional aids. This alternate to having each student experience the live demonstration could perhaps be applied to many other situations where live animals are used.

Advantages and Disadvantages of using Lower Vertebrates: There are several advantages to using these animals for testing, research and education. First, in general, they are small and plentiful. Second, they are inexpensive and require little space for maintenance. Third, because the frog is so similar to man from an anatomical and muscle structure perspective, they are excellent specimens for the study of the circulatory system, muscles and placement of organs.

The major disadvantage of using the lower vertebrates, lies in the fact that one cannot look at fetal toxicity which is transmitted from the mother's exposure to a toxin because the embryo develops outside of the mother. Consequently, there is no exchange between mother and fetus via the placenta. This limits the types of teratology studies that can be performed realistically.

4. Use of Lower Mammals (Especially Non-Primates)

The class Mammalia, in terms of nervous system development and evolution, is the most advanced of the vertebrate classes which include the Pisces (fish), Amphibia (e.g., frog), Reptilia (e.g., snake, turtle) and Aves (birds).

Among the significant differences of these classes are the following. The Pisces, Amphibia and Reptilia are poikilotherms or cold-blooded vertebrates. That is, their body temperature is unregulated and reflects the ambient temperature. The members of the classes Aves and Mammalia are homeotherms or warm-blooded vertebrates. They can maintain a constant body temperature despite fluctuations in ambient temperature. The circulatory systems of the vertebrates show an evolutionary progression from a two-chambered heart (fishes) to a three-chambered heart (amphibians and reptiles) to the most efficient arrangement, the four-chambered heart of birds and mammals which keep oxygenated (arterial) blood completely separate from non-oxygenated (venous) blood. Among the major changes in the evolution of the brain have been an expansion of the cerebrum and a reduction in the olfactory lobes. The former has been achieved by an increase in size along with the development of convolutions in the cortex (surface), thereby increasing the surface area. (This trend is accentuated among the homeotherms, and especially among members of the Order Primates, which includes man.

The cerebrum is the site of control or localization of many important activities including much sensory activity, muscle control, sex urges, mathematical reasoning, memory, and interpretation.

The mammals are grouped into three categories based on the birth pattern. The Protheria exemplified by the duck-billed platypus and spiney anteater are egg laying mammals. The Metatheria produce a placenta for exchange of gases, nutrients and wastes between the mother and embryo, but the young are expelled from the uterus before development is complete and wander into a pouch where development continues. The opossum and other marsupials, such as the kangaroo, exhibit this birth pattern. The Eutheria, exemplified by most orders of mammals, have embryonic development proceed to a later stage or to completion before the new individual is expelled from the uterus.

It is not within the scope of this report to describe in detail most of the significant differences and similarities among the vertebrates or mammals. It should suffice to note that the vertebrates, including the mammals, are heterogeneous in regard to body structure, physiology, adaptation, and biochemistry. The heterogeneity has lead to a "crazy-quilt" pattern of overlapping similarities as well as differences. The Class Mammalia has evolved into 18 orders whose separation is marked by pronounced evolutionary adaptations, such as the Cetacea to the sea, (e.g., sea lion, porpoise) the Rodentia (e.g., squirrels, rats) for exploitation of gnawing, and the Primates for stereoscopic vision, abstract thinking, and tool-making.

Because many of the significant characteristics of the Primates have a well-developed, or at least a rudimentary counterpart or analog in the "lower" mammals, the non-primate mammals may serve as useful models for testing, research and education. Their attributes include availability, shorter life-span, early post-natal maturation, and short gestation period. Furthermore, strains exhibiting desired characteristics, ranging from susceptibility to cancer to different levels of animal intelligence, can be developed by selective breeding and exploited for different scientific reasons. Of the various non-primate mammals, the laboratory mouse and laboratory rat are most widely used. Their use corresponds to about 84% of use of all laboratory animals (Rowan, 1984).

The use of such animals as models for an understanding of the human condition has proved to be a valid approach. Nevertheless, on occasion, problems arise when the extrapolations which are made are unrealistic or when the animal model and the human do not share,in kind or in degree, some common, important element. Thus, there are occasional miscalculations and inappropriate applications of animal models.

Use of Lower Mammals for Testing. One can obtain only approximations regarding how many lower mammals are used for toxicity testing and which species may be involved. Rowan (1984) estimates that 45 million mice, 15 million rats, 1 million hamsters, 1 million guinea pigs 750,000 rabbits, and 8,575,000 other animals, including 25,000 primates, are used annually for all purposes. It is estimated further that 20% of animal use is for toxicology programs. If we assume that all animals are used in the same proportion in toxicology and other programs, these figures translate into 9 million mice, 3 million rats and 200,000 hamsters, 150,000 rabbits and 1.7 million animals of all other species. This assumption is not unrealistic in view of the large percentage of mice and rats used for all purposes.

The percent use of animals in various kinds of toxicity tests may be approximated from data reported for the United Kingdom in 1977 (Rowan, 1984): acute tests - 55.8%; subacute or chronic tests - 19.9%; teratology tests - 2.9%; distribution and metabolism - 16%; more than one - 5.4%.

Use of Lower Mammals for Research. Rowan (1984) estimates that 40% of all laboratory animal use (28.5 million) is for research while 43.6% of the NIH research awards for 1980 involve projects that use non-human mammals (Scientists Center Newsletter 4 (1), March,1982).

The pattern of use of different kinds of animals and the underlying rationale reported above for testing also applies to research.

Research efforts may be basic or applied. The former has as its primary objective understanding while the latter has as its primary objective practical application for the more immediate medical, social, or economic betterment of mankind. Because of the differences in these goals basic research tends to be less rigid in its use of different kinds of animals, including exotic species, than applied research. The latter is locked into the use of traditional animal models which reflect the human condition. The basic researcher may use animals to uncover the evolutionary origin of a phenomenon, or to better understand adaptation to a specific environment, or to solve the riddles of migration by diverse species.

Use of Lower Mammals for Education. Mammals are used for education purposes in a variety of settings and for diverse purposes. These include anatomy, behavior, and physiology studies of mammals in general or the somewhat unique characteristics of different orders or species of mammals. They may also be used to attract and rivet the attention of the student or develop in them, over a period of time, the nuturing feelings and skill required for good animal care and handling. Many of the situations described above in the section Reduction in Numbers are appropriate for inclusion here.

Advantages and Disadvantages of Lower Animal Use for Testing, Research and Education. The critical issues in regard to testing with lower mammals rather than with sub-human primates are the relevance of the test to human exposure and the accuracy of the test. The latter is more easily evaluated than the former. The accuracy can be improved by improved experimental design, the selection of objective end-points, and groups of animals sufficiently large in number to provide the statistical reliability being sought. Relevance of the test often is a subjective determination. Not only are there differences between the way a particular chemical is absorbed, distributed, sequestered and excreted, in animals and humans, but the availability of the target may also be different. This species gap makes it difficult to accurately predict in advance all the inconsistencies which may arise between animal and human toxicity. The problem is exacerbated further by the differences between the animal exposure situation and the human exposure situation and the human exposure situation. The former is carefully controlled and entails a population on identical diets, housed in a common environment, and having very similar backgrounds. The human population is far more heterogeneous and variable in background and patterns of exposure. Hence, it is reasonable to assume that the tests which are apt to be most predictive are those which involve more than one species and in which the test chemical is "handled" in a manner similar to that in humans.

Because of the species gap, tests with sub-human primates are sometimes introduced. Because of their cost difficulty in handling, and longer life span, such tests involve fewer animals. Hence, at times a trade-off may have to be made between accuracy and predictability enhanced by more thorough testing using larger numbers of animals of a more distant relationship to humans. The decision often boils down to a cost-benefit analysis of one approach over another. The analysis revolves around trade-offs in accuracy, time, money, and risk intermeshed with the question of available facilities and other resources including animals. There is no pat answer regarding which is best. Only validation and retrospective analyses can provide the answer.

The analysis presented above for testing is germaine to research, although less so for basic research which is not directed at an immediate practical betterment of the human condition. Certain questions, such as those which reconstruct a phylogenetic relationship or an evolutionary trend or very narrow questions pertaining to a particular situation or species do not always have the opportunity to use models as surrogates. Depending on the species of interest, the investigator may be forced to use higher mammals or lower ones.

Except for a few very specific situations involving nervous system function and psychobiology, primates do not present sufficient advantages over lower mammals to warrant their use in most courses and programs. Indeed, in virtually all situations, the advantages of lower mammals, especially the rodents, provide sufficient diversity of structure, function, and behavior to assist in the meaningful education of students from elementary grades through university levels. However, although, the rodents provide great diversity, it is only a fragment of the total diversity of the Class Mammalia. Hence, such studies with rodents should be complemented by others involving members of other orders. This can be done effectively by coordinating the education program of a school with field trips to natural habitats, zoos and farms,

It should be recognized that another level of comparison which could be made, is this; a comparison between the use of lower mammals and humans for testing, research and education. Indeed, humans have been, used to advantage in each of these situations when non-intrusive, nonharmful, ethical approaches are used. However, advantages do not always accrue. Limitations in number, handling, background, and mental awareness may negate the usefulness of the human organism as an experimental or teaching tool. Again, there is no pat answer regarding the usefulness of any species.

5. Use of Invertebrates

Invertebrates constitute the majority of animal species, comprising over 90% of all the species inhabiting the earth. Accordingly, Kaiser (Kaiser, 1980) predicts that invertebrates are the future source of animal models for research and testing by the experimental toxicologist. Although their body structure is much less similar to man than are the vertebrates, their anatomy, physiology and biochemistry offer avenues for new approaches which have only been partially explored.

In order to reinforce the usefulness and broad applicability of the invertebrates as a group, it is appropriate at this time to cite specific examples of studies performed using these phyla. For the purpose of clarification, it is important to distinguish between 1. unicellular invertebrates, such as the protozoa, 2. organisms of cell accumulations without the development of true tissues, such as mesozoa and certain sponges, and 3. the majority of multicellular animals with true tissues, such as worms, insects and certain water organisms, which comprise the rest of the phyla. For a more complete taxonomic listing please refer to Table 3.

Use of Vertebrates for Testing, Research and Education. Protozoa are especially useful and offer several distinct advantages as subjects for testing, education and toxicological research for the following reasons:

(1) The protozoan cell is highly specialized in many cases, as in free-living organisms, but it may also be rather degenerate, as in certain parasites. Consequently, they offer opportunities for the easy investigation of a broad spectrum of cell-specialization related aspects. For example, the effect of toxins, such as phenols, on ciliary action can be performed (Banergi and Adal, 1973). It is possible to draw a parallel from protozoan cilia to human bronchial cilia, using smoke as a stimulus, because cilia and the biochemical control mechanisms are similar in the majority of organisms.

(2) A number of other protozoa, which are parasitic pathogens, have been extensively studied because of their medical importance as human or veterinary parasites, such as Entamoeba histolytica, Plasmodium malariae, Trypanosoma gambiense, etc. Hence the extensive information and vast toxicological knowledge available concerning these parasites can easily be extended to biological phenomena in other fields of experimental toxicology (Earampamoorthy and Koff,

Table 3. - Taxonomical Listing of the Invertebrate Animals

Phylum	Description	Representative Organisms
Protozoa	Unicellular	Amoeba Paramesian
Mesozoa (Metazoans)	Multicellular	Volvox
Porifera	Range from incipient tissues, w/o organs and interior cavities to multicellular tissues with organ systems	Sponges
Cnidaria (Coelenterata)	Radial mouth surrounded by tentacles w/nematocytes	Hydra
Ctenophora	Biradial symmetry, tentacles never encircle mouth, no nemertocysts. Some w/well developed mesoderm	Venus girdle
Platyhelminthes	Without anal opening and circulatory system	Flatworms Flukes Tapeworms
Nemertrans	With anaus, proboscis and circulatory system	Ribbon worms
Aschelminthes Class: Rotifera	Interior mouth, straight digestive tube, Posterior anus	Rotifera
Class: Nematode	Epidermal cords, only longitudinal body muscles, pharynx & excretory system	Round worms
Mollusca	Body mass covered by mantle secreting shell, coelom appears during development.	Chiton, snails, squids

(Table 3. - continued)

Phylum	Description	Representative Organisms
Annelida	Spacious mitomeric coelom, segmented bodies	earth worms leeches
Arthropoda	joint appendages, reduced coelom, exoskelton	crayfish grasshopper insects, fruit fly
Echinodermata	Secondary radial symmetry and water vascular systems	Sea urchins, Sea Cumcumbers
Chordata	Bilateral deuterostromes, adults with gill slits or vertebral column or both; notochord in embryo	Fish-like animals

3) For a given type of damage (e.g., point mutations), there is no one test which is entirely predictive. Hence, a number of tests should be incorporated into a test battery to increase the level of certitude regarding the mutagenic potential of a chemical. 4) The *in vitro* tests should allow for metabolic activation supplementations (Ames, 1971; Clive and Spector, 1975). 5) Tests should allow for the distinction between lack of genetic damage and damage which has been repaired.

The mutagenesis tests currently available are promising in that they allow for the detection of point and chromosomal mutations, they can be supplemented with metabolic activation systems, and they permit the assessment of DNA damage in the presence and absence of DNA repair capability (McCormick and Maher, 1977).

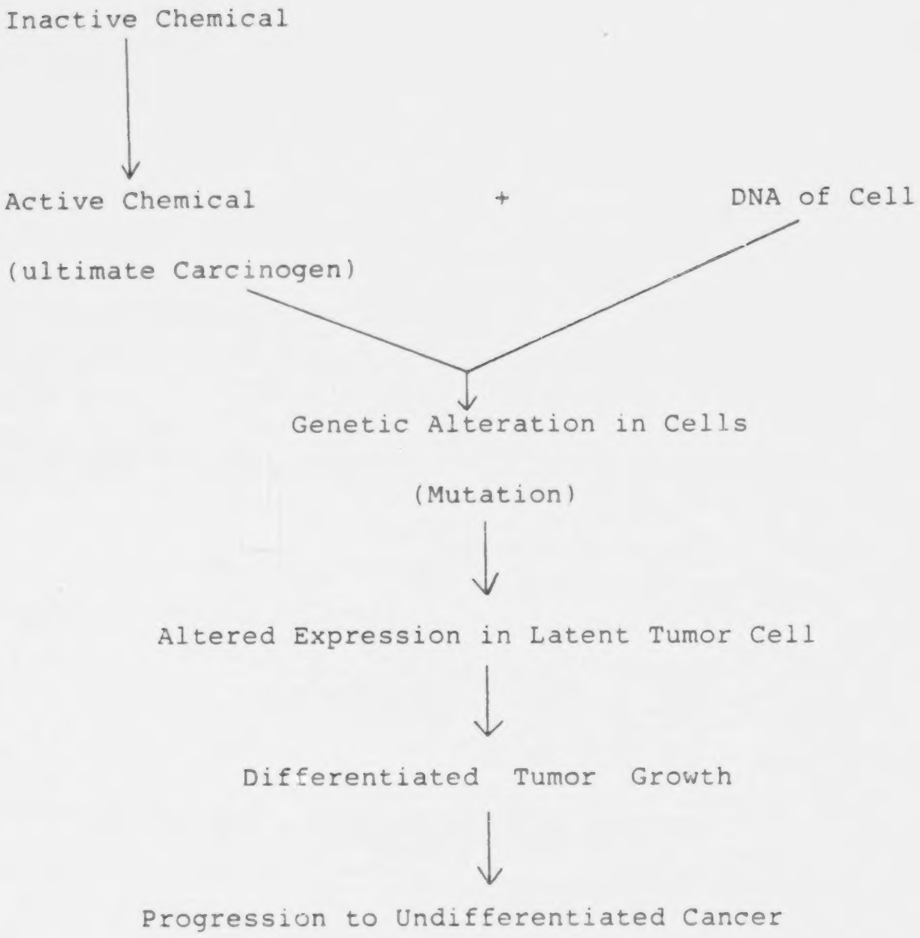
Carcinogenicity tests: The evaluation of *in vitro* tests for the detection of potential chemical carcinogens must be made in the context of a understanding of the carcinogenesis process in humans and other animals as a multistep process. This process has been described in detail by Weisburger (1976) and involves, in most instances the chain of events shown in Figure 1.

The progression from metabolic activation, through gene mutation, through tumor formation, through the metastasis of undifferentiated cancer cells is a process which can be reversed, blocked, attenuated, or accelerated at different stages, depending on the responses of the organism. For example, DNA repair can correct the gene lesion, the P450 enzyme level in the liver will influence the rate of metabolic activation, and the immune system may destroy tumor cells after they are formed while a defective immune system may accelerate the rate of cancer progression.

In view of the nature of the multistep process of cancer formation the ideal, complete *in vitro* carcinogenesis test system should have the following attributes. 1) It should permit metabolic activation of procarcinogens to carcinogens. 2) It should allow for the detection of cell transformation. 3) It should allow for the detection of cell transformation. 4) It should lend itself to the evaluation of tumorigenic and carcinogenic properties of the target cells. 5) It should allow for evaluation of promoters and cocarcinogens.

The test systems which are in current use have as their primary end-point cell transformation, i.e., the alteration of a normal cell to a modified cell which has some properties of tumor cells. These

Figure 1. - The sequence of events leading from metabolic activation to development of a cancer.


Inactive Chemical
Active Chemical
+
DNA of Cell
(ultimate Carcinogen)
Genetic Alteration in Cells
(Mutation)
Altered Expression in Latent Tumor Cell
Differentiated Tumor Growth
Progression to Undifferentiated Cancer

properties include release from growth control including absence of contact inhibition, growth in soft agar, and alterations in chromosome number. Cells showing these properties are designated as presumptive tumor cells and are then infected into an appropriate animal host in order to evaluate their ability to cause tumors. In other words, the in vitro tests can evaluate the mutagenic event leading to cell transformation, with and without metabolic activation.

The invitro cell transformation tests employ diverse cellular systems (Table 10). Most of these tests use fibroblasts from rodents such as mouse, rat, and hamster. The cultures of cells exposed to a test chemical are monitored for evidence of regions of multilayered growth because most transformed cells show the absence of contact inhibition and instead of growing as a single cell layer (monolayer), the cells grow on top of one another, a phenomenon which is readily detected. Such cells usually clone in soft agar and form tumors when they are injected into a susceptible test animal. Such studies can be performed with and without metabolic activation. Hence, one can determine with this test system whether or not a chemical can be changed from harmless to harmful. Also, such tests can be used in the evaluation of chemicals which may promote tumor formation.

The cell transformation tests (for potential carcinogenesis) are not as well-validated as the invitro mutagenesis tests. Nevertheless, sufficiently high correlations have been established between carcinogenesis in animals and cell transformation in vitro to warrant the National Toxicology Program to include these tests among those to be validated and used for limited testing (Zeiger, 1983).

Cell transformation tests, in the absence of coupling to animal studies, are not suitable for evaluation of the final progression to carcinogenesis nor are they suitable for the evaluation of many of the modifiers of the progression, such as immune influences.

Despite these limitations, the in vitro cell transformation tests can inform one of the initiating events. Hence, chemicals which initiate such events will be designated as potential carcinogens. Again, we see the great gap which exists between screening and risk assessment.

While human cells in culture have been used for mutagenesis tests (McCormick and Maher, 1977), their usefulness for carcinogenesis testing

Table 10. - Representative In Vitro Cell Transformation Test

1. *In Vitro* Transformation of Balb/c-3T3 Cells with S9 Activation
2. *In Vitro* Transformation of Balb/c-3T3 Cells with Rat Hepatocyte Activation
3. *In Vitro* Transformation of C3H/10T½ C18 Cells
4. *In Vitro* Enhancement of Balb/c-3T3 Transformation; Two-Stage Initiator-Promoter Model
5. *In Vitro* Transformation of Syrian Hamster Embryo Cells

is virtually non-existent. While human cells can be easily transformed with oncogenic viruses, they are very difficult to transform with chemicals (Milo and DiPaolo, 1978). This refractility may stem from a very efficient DNA repair capability or factors relating to oncogene suppression in human cells.

Cell Viability. Cell viability can be measured in several ways including by dye exclusion and radioactive chromium leakage from prelabeled cells. Reproductive death, that is, the inability of a cell to reproduce sufficiently to form a visible colony, is another commonly used method in cell viability studies (McCormick and Maher, 1977).

Cell viability studies, becuase they have an unambiguous end-point (live or dead) and because they deal with lethality, which is a major focus of animal toxicology studies (as in LD50 and other acute toxicity studies), are almost always included in in vitro toxicity studies. The tests are rapidly performed and are useful for the comparison or ranking of the toxicity of a variety of chemicals. Such in vitro ranking provides a basis for comparison with the toxic response of animals. Again, the limitations of in vitro systems cited above - lack of metabolic activation and limited repair capability - impinge on the interpretation of such comparisons. Furthermore, in many situations, death of an animal may not be attributable to cell death. For example, transmission enzyme impairment in a critical region of the brain, such as the respiratory control center, could lead to death of the animal despite the overall ability of the malfunctioning cells to remain alive. It is for this reason that differentiated cells have a special role in in vitro toxicology. Their activity and response to toxins under ideal circumstances, should be comparable to that of similar cells in the whole animal.

Another caveat worth noting is that the complexity of any tissue in an animal makes it impossible for any one cell culture system to reflect the entire tissue. Nevertheless, if cell culture toxicity studies could demonstrate unequivocally that a particular vital process or essential cell type would be damaged by a particular toxin, there is little need to know the possible effect on all other cell types in the tissue.

The lack of a circulating blood supply in cell cultures which can redistribute toxins and by-products of injured cells has promoted some investigators to explore the suitability fo whole chick embryos and

and the chorioallantoic membrane of embryonated eggs for toxicity studies (Leighton et al, 1983). These are not in vitro systems because cells or tissues are not being maintained extracorporeally. Their inclusion here is based on the fact that they have many of the advantages of tissue culture (accessability, cost, control) and fully developed sentient animals are not involved. The chorioallanotic membrane system mirrors, to a certain extent, the increased vascularization associated with irritancy such as occurs in ocular toxicity. Also, no pain is associated with its use. Leighton's studies (1983) in which a large number of known ocular irritants were screened, show a good correlation between irritancy of the rabbit eye and the response of the vascular supply in the chorioallantoic membrane. He is optimistic about the possibility of developing and using this assay as an alternative to the irritancy position of the Draize test. This is discussed in the section, Draize Eye Irritancy Test.

Use of Organ Culture in Testing: Organ cultures represent a major untapped system for toxicological evaluation. The reasons why this area of in vitro toxicology has been late to develop are myriad. Cell cultures are much easier to manipulate, to dose, and to evaluate with respect to several key end-points such as cell death. Furthermore, cells in organ cultures are often suppressed in their proliferation. Hence, a constant return to animal donors is necessary for protracted experiments. Furthermore, it has been only relatively recently that the ways to optimize survival and expression of differentiated function of a variety of organ cultures have been learned (Paul, 1976, Huot et al, 1981).

The difficulties attendent to the maintenance of organ cultures are associated with their thickness (many cell layers) coupled with the lack of a circulatory system which ordinarily distributes respiratory gases, nutrients, and toxic wastes. The introduction of raft techniques for the support of floating organ cultures, chambers which permit the maintenance of a high positive gas pressure and improved media with hormones, have made organ culture research very attractive. The applications of organ or organoid culture to neurobiology are illustrative of the value of such systems for studies on cell lineages in developing brain (Fedoroff and Hertz, 1977) and axonal pathways in slice cultures of the cerebellum and hippocampus (Gahwiler, 1984).

The development of automated perfusion systems for the regulation of pH, oxygen, carbon dioxide and nutrients, and which permit the continuous or pulsed delivery of chemicals to such cultures, offer an opportunity to more fully exploit such systems.

Two systems which have sophisticated sensors and controls are the IL410 manufactured by Instrumentation Laboratories (Lexington, MA) and the ACUSYST^{TM} SERIES manufactured by Endotronics (Minneapolis, MN). These systems, with appropriate modification could be used for organ cultures. The IL410 uses permeable teflon bags as containers for the biological material. As the nutrient medium is circulated through the bags, the medium has its oxygen and carbon dioxide levels determined by sensors, which in turn open gas tank regulators on an "as needed" basis to adjust the gas tension, and simultaneously the pH. The Endotronics units have controls interfacing with computer programs which specify the pattern of perfusion or introduction of materials into the tissue culture medium and patterns of collection of medium for cellular analysis or chemical characterization.

Use of Embryo Culture in Testing: Earlier, the potential usefulness of chick embryos was mentioned in the context of using the entire embryo, while it is still in the egg, as an experimental organism. The development of techniques for the maintenance in culture of whole mammal embryos is expanding the opportunities for whole embryo studies (Fantel, 1982). Embryos of chickens and other species such as hamster, mouse and rat have long been used as a source of primary cultures. Primary cultures are those which have been established directly from a donor and which have yet to be subcultured. Primary cultures may be established as primary cell, tissue or organ cultures. For the former, the embryos are treated with a chemical, such as trypsin, in order to separate the cells one from another. The resultant culture is then a mixture of cell types which no longer have the original spatial relationship one to another. The tissue culture environment will ordinarily favor the attachment, growth and reproduction of specific cell types. Hence, with passing time, the cellular mix will change and one or a few favored cell types will dominate. The culture will show progressive evolution and with each passing day the culture will resemble the original donor tissue less and less. Nevertheless, the behavior of the cells which dominate will be more like that of their counterparts in the body than will normal diploid or tumor cells which are serially subcultured. This is well illustrated by the fact that primary hamster embryo cultures are capable of metabolic activation of toxins, but such activity is markedly reduced or absent in cultures which have been passaged two or three times (Kouri and Schectman, 1977).

Primary tissue and organ cultures of embryos are used because the normal tissue architecture exists. Their usefulness, as well as that of primary cell cultures, varies. It should be borne in mind that the cells will reflect the stage of embryonic development. Hence, many immature or undifferentiated cells will be present. These cells tend to survive and proliferate rapidly in culture. On the other hand, their developmental stage may make them unusually sensitive to exposure to toxic chemicals and their frequency may not reflect the situation of a post-natal organism. Their potential usefulness for toxicology studies which focus on teratogenesis (embryonic malformation) is self-evident (Kimmel et al, 1982) and will be discussed later.

Human fetal material, obtained as products of spontaneous or induced abortions, has been a widely used source of primary cultures of human material. Their attributes and limits of their usefulness is similar to that specified above. However, ethical concerns as well as limitations on their availability and lack of suitable control during the collection process has restricted their use (Joanne, 1977).

Teratogen Detection: Teratogens are physical or chemical agents which cause embryonic malformation. Such malformation may lead to embryonic death or the birth of malformed individuals. Death of embryos (termed fetal or embryonic wastage) may be due to the direct effect of the agent on the embryonic cells and tissues or it may result from effects on maternal physiology which, in turn, may result in abnormal embryonic development (Wilson, 1974). At times toxicological evaluation for teratogenic effects is pursued independently and at times as part of a reproductive assessment program. The latter, includes the effect on sperm and egg formation, behavioral phenomena (such as courtship patterns and sex drives), post-natal nurturing and other factors. Wilson (1974) has identified at least 13 episodes which should be part of reproductive assessment with teratogenesis being only one part. The following evaluation addresses teratogenesis only.

A number of studies (Wilson, 1974; Brusick, 1978) have demonstrated a very strong correlation between mutagenesis, carcinogenesis, and teratogenesis. Although the correlation between mutagenesis and carcinogenesis, on one hand, and terotogenesis on the other, is not as great as that for mutagenesis and carcinogenesis, the good correlation leads one to suspect strongly that many teratogenic events may stem from modification of the expression of genes--either as mutations or as non-transmitted modifications of gene expression. Indeed, one may reason that alterations in genetic information will be expressed as alterations in development patterns which will lead to wastage and malformations.

Because developmental events are under tight temporal control, adverse chemical effects on early developmental stages will have consequences which will be amplified during development. Hence, if one cell of a two-cell embryo has a genetic change, that change will be transmitted to all the cells which will descend from that cell. This may mean that half of the cells of the embryo may be affected. In animals the period of organogenesis, when the major organs are developing, is the most sensitive to toxic chemicals. For humans, this is the first trimester of development.

Animal models for teratogenesis include the mouse, rats, Syrian hamsters and ferrets (Collins, 1978; Wilson, 1975). The latter is employed because its pattern of placenta formation resembles that of humans more closely than that of other experimental animals. In animal testing, the agent is usually administered to the pregnant animal and the effect on embryonic wastage and the formation of terata is scored. Thus, the maternal chemical metabolism (activation and detoxification) and transplacental transport affect the outcome.

In a typical evaluation of a chemical, animals at various stages of pregnancy are administered differing doses of the test chemical. Some animals are sacrificed at various times during pregnancy and the uterine contents examined while other pregnancies are allowed to go to term. A typical evaluation using mice involves ten males and thirty females for each dose level.

In vitro systems are being used for teratogenesis studies, primarily for mechanistic ones. However, in recent years attempts have been made to use cell, tissue and organ cultures as well as whole rat embryo cultures for the screening of teratogens. Because teratogenesis is so complex, involving maternal as well as fetal factors, and because the state of the art of _in vitro_ test development for teratogenesis is very primitive, Wilson and Nardone (1975), in a position paper, pointed out that _in vitro_ screens for teratogenesis testing are not ready for wide-scale use at this time.

Despite the current situation, progress is being made and in selected situations in vitro systems could be used in decision-making and in the monitoring of human populations. The latter is being pioneered by Klein who is screening human subjects who have been on medication, for a long period of time (Chatot et al, 1980). The patients' sera is used to supplement tissue culture growth medium for the maintenance of whole rat embryos in culture. Reduced growth of embryos or malformations are indicative of toxic levels of the medication in the serum.

Use of Cell, Tissue and Organ Culture for Research: The explosive growth of in vitro research during the 1960s and 70s is illustrated by the following. The Index of Tissue Culture had 84 pages of entries in 1965, 207 pages of entries in 1970, 566 pages in 1975 and 636 pages in 1980 when publication was discontinued because computerized information retrieval was warranted. There is virtually no field of biology or medicine which has not been affected by in vitro technology or felt its impact. In vitro models for the study of cell senescence, artherosclerosis, development, growth, and immune reactions are illustrative of the diversity of research applications. The Human Genetic Mutant Cell Repository and The National Institute of Aging Repository at the Institute for Medical Research, Camden, N.J. has about 7,000 different cell lines from normal donors and donors with genetic disorders. These were deposited in the collection in slightly more than a decade.

This growth stems from the technical advances which provided researchers with the unique opportunity to study isolated body parts (cells, tissues, organs) under carefully controlled conditions which could be modified as needed. The growth of the discipline is proof of the value of in vitro systems.

Table 6, which summarizes the characteristics of in vitro systems, specifies that as organization is disrupted or lost (as with cell cultures), the in vitro system has less and less of the kind of intracellular interaction which characterizes cells in the body. Nevertheless, improved accessibility of added chemicals and the opportunity to achieve genetic homogeneity by cloning and genetic manipulation by selection and fusion are important trade-offs. Indeed, at times cell to cell interaction may interfere with an experimental objective.

Use of Cell, Tissue and Organ Culture for Education: Because *in vitro* testing and research are so important, educators are trying to introduce the subject in their courses. For a student, there is no more convincing proof that cells from an animal have retained a significant level of autonomy than to have them isolate and culture cells.

Such cultures can be used to demonstrate a variety of principles relating to growth, development, cell cycle kinetics, and gene expression. However, the specialized training and facilities required to do this effectively far exceeds the available resources at most education institutions. Furthermore, most of these principles can be learned using other models which require less money.

Special In Vitro Opportunities:Continuous Cell Lines and Perfusion Systems. Newly established cell cultures may include cells which are so specialized that they no longer proliferate while others will proliferate for a limited number of population doublings before they age and die (Hayflick and Moorhead, 1961). Hence, a new animal donor will be needed each time a new primary culture is to be established. In some instances, however, transformed (tumor) cell lines could be used to great advantage. Some of these cell lines, in addition to having the attribute of immortality in culture, have the attribute of expressing some of the essential characteristics of the normal, specialized cell which is their counterpart. For example, glial cells of the nervous system are cells which support the neurons. A glial tumor cell line - a glioma - expresses in culture the specialized property of S-1 protein production. Hence, S-1 protein production should be considered as an end-point for evaluation of toxicity to glial cells in culture with glioma cells serving as the target. Other examples include antibody production by myeloma cell lines which are circulatory system tumors, hormone production by tumors of endocrine cells, hemoglobin production by Friends leukemia cell line, and the neuronal end-points cited for neuroblastoma cells in Table 11.

Perfusion Systems. The ability to perfuse a culture, that is, have the growth medium circulate rather than remain static, has been used successfully with cell cultures as well as organ cultures (Kruse and Patterson, 1973). Perfusion conditions present an opportunity to provide the cultures with a more uniform chemical environment. Also, under these conditions, cells tend to grow as a multilayer rather than as a monolayer. Thus, a higher cell density per cm^2 of growing surface can be achieved. Knazek, et al (1972) used a hollow fiber perfusion system for the growth of tumor cells which have the ability of producing the choriogonadotrophic hormone. They found the yield of hormone to be high. The system also facilitates the periodic collection of samples for assay.

Perfusion systems have the potential of being very useful for *in vitro* toxicology because the circulating medium can be readily modified to imitate the patterns of toxin distribution and change which occurs in the body. For example, a tandem arrangement could have the medium containing a xenobiotic circulate over a hepatocyte culture where it will be metabolically activated and then circulated through a culture containing the target cells. With the advent of computer controls of scientific equipment, such as pumps and gas regulators, and with the development of on-line automated assays, such systems should become popular (Peluso and Gruenberg, 1983).

Advantages and Disadvantages of Cell, Tissue and Organ Cultures for Testing, Research, and Education: While it is obvious that life in a test tube is under conditions different from those in the body, it is not always obvious what are the consequences of such a change in habitat. A few have already been cited. The medium is artificial and only mimics body fluids in a superficial way. Cell to cell interaction is diminished. Gene expression may be modified. The diploid cells undergo aging. The continuous cell lines are heteroploid with the members of the population differing in their chromosomal and genetic composition. The substratum for attachment is artificial. Populations evolve as the handling of cells or their environment is changed. The quality control measures to be implemented are rigorous. Sterility must be maintained. The investigator must submit to the culture's timetable. The work is expensive and labor intensive.

Balancing this is the opportunity for control manipulation; - a worthwhile trade-off.

Additional attributes and limitations for testing are described in subsequent paragraphs.

Many environmental chemicals are innocuous in their native state but become toxic when they are metabolized within many different sites of the body including the liver, kidney, testes, skin, and lungs. Such metabolic activation and its opposite counterpart, detoxification, influence the toxicity of chemicals. The net toxic effect is related,

Table 11. - A Proposed Test Battery for Neurotoxicity Testing

Components of the Test Battery:

1. Mouse neuroblastoma NIE-115
2. Neuroblastoma x glioma hybrid cell line NG108-15
3. Chick brain cell aggregate and cortex organ culture
4. Mouse primary spinal cord culture
5. IMR-10 human diploid cell line

Neuronal and Non-neuronal End Points Used in the Test Battery

Neuronal End Points:

1. Neuron-specific enolase activity
2. Neurotoxic esterase activity
3. Acetylcholine esterase activity and localization
4. Choline acetyltransferase
5. Tyrosine hydroxylase
6. Microtubular organization in neurites
7. Vesicles in neurites
8. Brain aggregate topography and neuronal and glial cell distribution

Non-neuronal End Points:

1. Cell viability
2. Plating efficiency
3. Cell proliferation
4. Macromolecular content and synthesis rates (DNA,RNA, and protein)
5. ATP levels
6. Total enolase activity
7. Total esterase activity

in part, to the total activation, detoxification, excretion, storage, and distribution response of the whole animal. Hence, any in vitro system which is to be used for the evaluation of the potential toxicity of a large variety of chemicals, must have this capability. Unfortunately, only limited progress has been made in getting a variety of cell culture systems to maintain metabolic activation capability in culture (Kouri and Schechtman, 1977). Most success is achieved when cells in culture are complemented with liver fractions (microsomes or S9 preparations) or cocultivated with hepatocytes. Although progress in this area has been significant, it should be remembered that such culture systems possess only a portion of the diverse metabolic activation systems of a whole organism.

A comparable limitation exists with respect to repair of cellular and tissue damage. Repair within the body of a whole animal is mediated at many levels - molecular, cellular, and tissue - and often involves the interaction of diverse cells from different tissues. Some genetic damage can be repaired by cells in culture (McCormick and Maher, 1977) but many other types of recovery are beyond the capability of cells in culture. Another limitation of in vitro systems for toxicity testing is that natural barriers within a whole animal limit access of some chemicals to selected tissues or organs. The blood-brain barrier and the blood-testes barrier are good examples.

These three factors - metabolic activation and repair limitations and blood-tissue barriers - contribute greatly to the difficulty in using tissue culture systems for risk assessment, which is the most important aspect of safety evaluation. However, they are very useful in other roles. These are screening, mechanisms studies, and personnel monitoring (Nardone, 1981).

2. Microorganisms:

Until recently, interest in micro-organisms was primarily restricted to studying them in relation to their activities as causal agents of disease. This subject is called bacteriology or microbiology. A distinction should be made between prokaryotic microorganisms and the single-cell invertebrates discussed earlier. The prokaryotes are single-celled organisms without a true nucleus and are characterized by the presence of a cell-wall and belong to the kingdom Monera.

In 1971, Ames (1971) developed an in vitro assay for detecting the toxic and or mutagenic effect of compounds by utilizing certain microorganisms. This was an important development because it offered animal alternatives for making such determinations.

It is important at this time to clarify the relationship between mutagenicity and carcinogenicity. A substance is a mutagen when it causes an alteration or damage to the DNA of a cell. The damage may or may not be permanent. If permanent, the mutation will be passed on to all the progeny of the affected cell. Certain types of mutations can result in cancer. Carcinogenicity, on the other hand, is the product of a complex series of reactions which result in cancer. It is thought to begin by some type of biochemical activation of a procarcinogen to the stage of ultimate carcinogen. The interaction of such ultimate carcinogens with specific cellular and molecular receptors, mainly DNA, results in altered DNA. Finally, the cells containing the abnormality multiply until they develop into a well differentiated tumor. Mutagenicity assays are widely used because if a compound is capable of causing a mutation, it is a potential carcinogen as well.

Use of Microorganisms in Testing: The mutagenicity assays developed by Ames have been widely used because they offer rapid and inexpensive means of acquiring information that is useful for selecting and ranking chemicals to be submitted in long-term animal carcinogen assays, thereby reducing the number of chemicals tested in animals. Basically, the test utilizes 4 strains of Salmonella typhimurium for detection of frameshift or base-pair substitution reverse mutations (Ames et al., 1973a, Ames et al., 1975 and McCann et al., 1975a).

This is accomplished by spreading 10^8 bacteria evenly over an agar surface lacking histidine. The bacteria used are mutant strains of Salmonella, which are unable to synthesize histidine, thereby rendering them incapable of growing in its absence. A small paper disk, containing the test chemical, is placed in the center of the petri dish to allow it to diffuse out into the agar. If the chemical is a mutagen it will cause a frame shift in the mutant bacteria making it possible for them to survive and grow in the absence of histidine. This is known as a reverse mutation. The number of bacteria reverted back to normal are measured by counting the revertant colonies on the plate after 2 days incubation.

Because many chemicals require some tupe of metabolic activation to be toxic, tissue homogenates (S9 mix) are used to convert inactive chemicalss to their active mutagenic forms (Ames et al., 1973b).

Liver homogenates are most often the source of activation enzymes.

However, several other metabolic activation pathways have evolved as well as various additions to, and modifications of, the "S9 mix". For example, various dietary amines can be activated to nitrosamines by incubation with nitrite in simulated gastric juice (Endo et al., 1977).

Subsequently, the original Ames test was incorporated into a hierarchical or tier approach because of the realization that the information gained by the use of bacteria alone was not sufficient to determine if a substance was in fact non-toxic or perhaps was giving a false negative result. Consequently, tier one added the yeast *Sacchromyces cerevisiae* to measure gene conversion or mitotic recombination. This organism was added to provide information on chromosomal effects. The second tier moves from single cell eukaryotes (such as yeast) to multicellular and eukaryotes such as *Drosophila melanogaster*, as well as the rat dominant lethal and the mouse lymphoma L5178-y tests. The final tier is to do *in vivo* determinations to detect major genetic damage, and determine dosages that are lethal.

The validity of the *Salmonella* /Ames test is quite high. It has been shown to detect all of the known organic chemicals that are human carcinogens except bezene and diethylstilbestrol. In several extensive studies designed to examine the ability of the *Salmonella* test to detect chemicals carcinogenic or non-carcinogenic in animal cancer tests, on the order of 90% of over 200 carcinogens tested were positive (McCann et al., 1975a; McCann and Ames, 1976; Sugimura et al., 1976; Heddle and Bruce, 1977; Purchase et al., 1976). The *Salmonella*/Ames test is one of the few short-term tests in which a large number of carcinogens and non-carcinogens, have been tested systematically using defined protocol. The fact that such a thorough analysis has turned up no clearcut examples of false positives in the test, indicates that the predictive value of the *Salmonella* test as a screening assay for carcinogens is a good one and also suggests that other tests that measure mutagenesis will be reliable predictors of carcinogenic potential.

The Ames test, however, has received some criticisms with respect to how legitimate it is as a screening test for two reasons. One is that the *in vitro* metabolic activation system used does not accurately reproduce the *in vivo* metabolism of the whole animal. Thus certain chemicals may not be activated to their ultimate carcinogenic state and may go undetected. Secondly, bacteria are so different from mammals that the information obtained is not directly applicable to human safety evaluation. Finally, the Ames Salmonella assay could fail to detect some mutagens because it is an assay for reverse, rather than forward mutations.

Despite all the theoretical arguments that can be raised against the Ames test, it remains the most widely used method for screening chemicals for potential carcinogenicity. There are several reasons for this. First, there have been several independent large-scale studies on the correlation between mutagenicity in Salmonella and carcingenicity in mammals (McCann et al., 1975b; McCann and Ames, 1976; Commoner et al., 1976; Sugimura et al ., 1976; Purchase et al., 1978 and Simmon, 1979). In addition, the Ames test is relatively rapid, simple and inexpensive to perform. Futhermore, the Ames system has accumulated a considerable data base which is available for comparison. For example, it is known to be good for detecting aromatic amines and nitro compounds, polycyclic hydrocarbons and direct alkylating agents as mutagens. Finally, the Salmonella system is very amenable for testing all types of compounds, whether they be gas, liquid or solid, because there is a sufficient amount of experience with respect to modifications of the test procedure.

It should be pointed out that the same basic procedure developed by Ames to study revertants in Salmonella has been applied to mutagen screening using mutant strains of E. coli and B. subtilis, Reverse mutations in E. coli have proven to be especially useful, because of the low frequency of spontaneous revertants, compared to Salmonella. Consequently, these organisms have proven to be useful for detecting weak alkylating agents (Hollstein and McCann, 1979).

Experimental systems which are designed to evaluate the mutagenic potential of a given agent to man require genetic tests that cover a wide range of effects, rather than just reverse mutations. Therefore Fungi have been added to the comprehensive battery of tests because of the wide variety of adverse effects to the genome of man that can be created by mutagenic agents such as point mutations, chromosomal structural aberrations and aneuploidy. However, tests commonly used in environmental mutagenicity screening are based on reactions that result from chemical alterations induced directly in DNA or interfere with DNA metabolism (Haynes et al., 1981).

Because proper segregation of chromosomes requires an intact spindle fiber, and in this case the molecular target and essential component is tubulin, tests based on the induction of point mutations or on differences in DNA-repair systems between normal cells and repair-deficient derviatives need not reveal the mutagenic activity of chemicals that induce only chromosomal malsegretation because they interfere with tubulin but not with DNA. Consequently, specific tests for chromosomal malsegregations have been designed utilizing fungi. However, the use of fungi in mutagenicity testing requires exogenous sources for metabolic activation. This was recognized very early, and the first use of mouse liver microsomes was reported by Malling in experiments with Neurospora crassa (Malling), 1979). Since that time, a wide spectrum of sources of exogenous activation have been combined with fungal mutagenicity test systems (Zimmerman et al., 1982).

The importance of utilizing fungi in a comprehensive mutagenicity testing battery is well documented by two recent reports. First, Parry and associates tested a number of tumor promoters and related substances for induction of mitotic aneuploidy in yeast (Parry et al. 1981). This strain allows simultaneously the detection of point mutation, mitotic recombination and mitotic chromosome loss. Several tumor promoters, one of which waa 12-0-tetradecanoylphorbol-13-acetate, were possible only for the induction of mitotic aneuploidy but not for any other type of genetic effect. Most interesting was the induction of mitotic chromosome loss by two fatty acids, lauric and oleic acids. It is most likely that the fatty acids cannot directly alter DNA; however, they may very well interfere with the integrity and function of the cellular membrane systems (Hoover et al., 1981). The second major contribution was made by Gualandi and Bellincampi based on the use of a strain of Aspergillus nidulans which monitors the full spectrum of genetic eukaryotic changes (Gualandi and Bellincampi, 1981). The authors tested known membrane active chemicals like ethanol, amphotericin B, and nicodazole, which interferes with the spindle fiber apparatus, as do benomyl and isopropyl-3-chlorophenyl carbamate (Oliver et al., 1978). Similarly, there was a specific induction of mitotic aneuploidy but no other type of genetic effect. Consequently, both reports document clearly that there are agents that would be missed in standard genetic toxicology testing based on effects induced directly in DNA or indirectly by interference with enzymes of DNA metabolism. It is precisely this type of situation that makes fungi such an important component of a testing battery.

In summary therefore, mutagenicity assays (in particular, prokaryotic test systems) offer a rapid and inexpensive means of acquiring information that is useful for selecting and ranking chemicals to be submitted to long-term carcinogen bioassays. However, whether mutagenicity and other short-term assays, singly or in combination, will eventually acquire the status of long-term animal tests in predicting human hazards depends entirely on further demonstrations of their ability to produce results that are consistent with those obtained in comparable animal tests as well as those obtained in adequately conducted human epidemiological studies, (Bartsch, 1983). One of the shortfalls of in vitro carcinogenicity and mutagenicity tests is that they often are qualitative rather than quantitative. Only in limited situations can they be used for specific quantitative ranking. However, in order to do risk assessment studies, quantitative data are necessary.

Use of Microorganisms in Research and Education. Prokaryotes and unicellular eukaryotes have a distinct place in research and education. For the most part, metabolic pathways have been determined through studies utilizing certain bacteria. Futhermore the wide variety of naturally occuring mutants of these organisms has provided the scientist with a naturally occuring resource for screening potential mutagenic and toxic substances.

Basic microbiology and all of the advanced courses related to this subject have been part of the university curriculum for years. This topic is extremely helpful for students to actually visualize the different growth patterns of the different types of microorganisms and makes metabolic pathways much more understandable.

With the onset of recombinant DNA technologies, a wide variety of microorganisms have been utilized to manufacture certain necessary enzymes, such as insulin, in large volumes. Understanding the molecular events that take place when a cell is infected by virus has been greatly enhanced. Furthermore, there is great potential for industry to utilize this technology for manufacturing certain metabolites in volume less expensively as well as more efficiently. There is also the possibility that these necessary enzymes, such as insulin, will be manufactured by a human chromosome in a human cell. The possibilities are enormous and they have come about by using microorganisms to get a handle on these techniques.

Advantages and Disadvantages of Using Microorganisms. 1) They reproduce rapidly at body temperature; 2) Rapid division (every 20-30 minutes) makes them useful for short-term monitoring; 3) Can perform studies, through many generations, in a short period of time; 4) Inexpensive in terms of storage, upkeep and maintenance.

The major disadvantages stem from the fact that these are unicellular organisms and consequently one cannot study the interaction of cells but rather the molecular structure of cells. Furthermore, if a toxic substance is to be properly tested it must first be exposed to some type of activation system because these organisms will not metabolically activate a pro-carcinogen

3. Plants

Use of Plants in Testing. The plant kingdom is often overlooked or not considered as a logical alternative to animal experiments. However, plants have proven to be very useful in education, research and testing. For example, plant cell culture has been used increasingly to study the effects of different plant viruses and infections in the laboratory. The data that has been compiled from these studies has served to help the agriculture industry with respect to the care and handling of plant contaminants.

Plants were utilized as early as the late 1950s by the Brookhaven National Laboratory's Biology Department as indicators of environmental mutagens. Initially, certain hybrid clones of *Tradescantia* were used primarily to study the effects of radiation on living organisms. The high sensitivity of this plant to physical mutagens made it a likely candidate as a test system for assessing the influence of chemical mutagens on living organisms. Recent laboratory experiments involving exposure to various chemical mutagens have produced very positive results. Currently, flower color mutation is being used as an indicator of potential health hazards resulting from natural and industrial pollutants.

Tradescantia is sensitive to a wide variety of stresses, both physical and chemical. Its color genes will respond to radiation and to many different kinds of chemicals, as well as to relatively large changes in temperature and light. After exposure to various forms of pollution, mutations are likely to occur in the color genes of the buds, actively dividing petals and stamen hair cells. They are heterozygous for flower color, and the blue gene is dominant over the pink gene. Therefore, normal unaffected plants will have blue flowers while cells containing mutated dominant color genes may express only their recessive pink character; thus, the petal and stamen hair cells will appear pink.

Tradescantia stock plants have been used since 1976 to assess the possible mutagenic effects of common pollutants by exposing them to ambient air at industrial sites all over the country. Trailers specially outfitted with elaborate air filtering and control equipment are used. The trailer contains three environmentally controlled chambers to support the growth of the plants during exposures. During testing, one of the chambers is supplied with filtered air to serve as a control, one is connected to a four-inch glass intake duct which pulls in polluted air from the outside, and one serves as a back-up unit in case either of the other two fails. After a given exposure time the cuttings are brought back to the lab and the pink cells are counted as the flowers bloom each day.

The plant Tradescantia appears to be well suited to air pollution work for several reasons. First, it measures the actual biological effects of chemical mutagens on a living organism, whereas, conventional monitoring systems measure only physical quantities of certain air pollutants. In addition, they often ignore the possibility of synergistic response, or pollutants mixing and interacting to cause effects that are greater than the sum of the effects of individual ones. Third, while conventional air pollution surveillance systems measure the level of specific, identified pollutants, the Tradescantia system may reveal the presence of previously undetected pollutants.

Use of Plants in Research and Education: It is important to bear in mind that algae, molds and fungi all belong to the plant kingdom and all of these organisms have been used in testing, research and education. Certain dinoflagellates (algae) produce a toxin that is extremely lethal to many fish and man. These organisms are grown in the laboratory and the toxin is then isolated and purified. Tests can then be done to characterize the toxin and research can be conducted on trying to produce an anti-toxin.

The slime mold has been used in junior high and high school to demonstrate peristalsic movement. It provides a very vivid example of movement by contraction that can be seen clearly and in color by viewing the slime mold under the microscope.

The discovery of pennicillin should not be overlooked either. Flemming made this observation when his bacterial cultures became contaminated with mold and caused an area of growth inhibition due to the production of the antibiotic by the mold.

Yeast, especially Saccharomyces cereveciae, have been used in research for many years. It is possible to study metabolism in these organisms in the presence of different toxins because the growth properties of the mutants differ from those of the wild types.

Furthermore, there are certain investigators who claim that a greater understanding of the process of cancer may result from studying the factors that control normal growth in plants. Knowledge of the molecular events which control normal cells might clarify how cancer cells are altered and deregulated.

Advantages and Disadvantages of Using Plants: One advantage of using organisms from this kingdom stems from the fact that they lack anything that resembles a nervous system, and therefore presumably do not feel pain. Consequently they would appear to qualify as good potential alternatives. Secondly, they have been shown to be susceptible to certain poisons and environmental pollutants that also affect man. Therefore, they should be used as model test systems whenever possible. Third, they are relatively easy and inexpensive to propagate. And finally, with the advent of plant cell culture, many more avenues of opportunity should be forthcoming and exploited.

The disadvantages arise from the fact that a number of biologically active substances which are toxic to animals are harmless to plants. However, this should not serve as a deterrent because a great deal of useful information has already been obtained by utilizing plants. For example, Mendalian genetics is based on studies that were carried out with plants. This data proved to be valid in the animal kingdom because there is a close resemblance in the genetic mechanism between plants and animals. A more recent attestment of that fact can be found in the work of a recent Nobel Laureat Barbara McClintoch. Her contribution to our understanding of genes and the movement of genetic elements was all accomplished by studying pigment segregation in maize kernels (Lewin, 1983).

C. NONLIVING SYSTEMS

The possible use of non-living (physicochemical) systems as alternatives to testing with animals offers little opportunity at this time. However, one can envisage that the current technology for the manufacture of liposomes and other artifical membranes (Bangham et al, 1974) and for synthetic nucleic acid sequences may have limited application at some future date, especially for research and teaching.

Lipsomes are chemically defined phospholipid vesicles. As such, they have physico-chemical properties of membranes. Their structure can be dictated by the investigator who can combine lipids and proteins of different types and in different ratios. The barriers formed by liposomes often are selectively permeable thereby increasing the popularity and models of biological membranes. Liposomes can also be used to encapsulate chemicals, such as drugs and nucleic acids, and serve as a delivery system of the chemical in a protected environment (Fraley et al, 1980). Thus, they lend themselves to basic studies on membrane damage and provide a vehicle for the controlled introduction and release of toxins into cells.

The code of synthetic nucleic acids can be specified in the manufacture of small nucleic molecules. Thus, one can envisage research projects which try to establish the relationship between specific base sequences in nucleic acids and the sensitivity or resistance of such nucleic acids to mutagenic and carcinogenic chemicals. Again, such studies would be research efforts geared to understand the causes and mechanisms of damage to DNA.

D. LD-50 Test and Possible Alternatives.

The LD_{50} test is a widely used animal test for the evaluation of the acute toxicity of chemicals in animals. The LD_{50} is that dose which is lethal to 50% of the test population, usually 60-100 animals. It is used in a variety of contexts ranging from preliminary experiments in carcinogen bioassay, to drug evaluation for a margin of safety (therapeutic index), to the toxicity ranking of chemicals. Furthermore, it may be called for by regulatory agencies as part of the safety evaluation programs (Page, 1983). The applications of and the range of challenges to the LD_{50} are reflected in a series of papers presented at the First CFN Symposium (Lindgren et al 1983).

The objections to its use include a call for a more complete testing program than is provided by the LD_{50} test to its modification or abandonment because it is misleading in its precision (Rowan, 1983).

The possibility of using tests with fewer animals and reduced precision, such as the Approximate Lethal Dose (Deichmann and LeBlanc, 1943) is advocated for some situations while tests which emphasize non-lethal toxicity are advocated for others.

Nardone (1983) has proposed a strategyfor the development of in vitro alternatives to acute toxicity tests in selected situations, such as when the Approximate Lethal Dose (ALD) is appropriate. The strategy is based on the following: 1) Cell culture screening tests show a good correlation with animal toxicity in selected situations. 2) Such tests, if properly validated, could be predictive of acute toxicity in animals. Hence, chemicals which fail to pass the screen may not warrant testing in animals or they may warrant an ALD study with a small group of animals.

A strategy for in vitro test validation is described in the section Validating alternative Methods.

E. DRAIZE EYE IRRITANCY TEST.

The Draize test is an acute ocular exposure test, usually performed on a few non-anesthetized rabbits, which monitors for effects on the cornea (the end-point is opacity), penetration into the eyes anterior chamber (iritis) inflammation (conjunctival hyperemia, chemosis, and discharge), and healing. The test has been under attack by Animal Rights groups because of the pain animals must endure when highly irritating chemicals are used and because the results may vary fromtest to test.

The politicization of this issue has prompted Nardone and Bradlaw (1983) to write ....

> "Attracted by the apparent success in the field of genetic toxicology in which bacteria and mammalian cell cultures are used to test for potential mutagens and carcinogens, organizations concerned with the reduction or elimination of animal use for toxicity testing initiated an intensive, well coordinated campaign to promote the development of _in vitro_ alternatives to the Draize test for ocular irritancy. The intensity of this effort is illustrated by the following. Resolutions for support of research in this area have been introduced in committees of both Houses of Congress. With encouragement from several members of Congress, the NIH sponsored a bioassay conference to examine alternatives. The Cosmetic, Toiletry, and Fragrance Association (CTFA) and Revlon have donated large amounts of money to establish alternative research activities at Johns Hopkins University and Rockefeller University, respectively. Several investigators have received grants from antivivisection groups to develop _in vitro_ alternatives for ocular toxicity testing. Similar efforts have been made in Europe.

Sufficient advances in ocular tissue culture and in the development of _in vitro_ tests for irritancy (Leighton et al, 1983) has suggested that _in vitro_ alternatives to the Draize test could be developed for selective use.

Nardone and Bradlaw (1983) cite a number of fragmentary validation studies which are promising. Two which warrant special mention are those of Stark et al (1983) and Leighton et al (1983,1984). In the former studies, known human ocular irritants and chemicals whose Draize test performance is known are tested on cell cultures for alteration in chemotaxis by macrophages, cell viability, membrane transport, and cytological alterations. In studies involving a series of alcohols a good correlation was seen when in vitro effects were compared with level of ocular irritancy.

The approach being used by Leighton et al (1983,1984) to evaluate potential irritancy entails the use of the chorioallantoic membrane of a developing (14-day) chick embryo as the test material. This membrane is a thin transparent vascular membrane with no demonstrable nerve fibers for the sensation of pain. an embryonated egg is easy to maintain, the membrane is easy to visualize, and it is inexpensive (three fertile eggs cost about $1.00). The system has the advantage of having functional blood vessels, an attribute not characteristic of cell culture test systems. Macroscopic lesions are accompanied by destruction of the blood vessels and necrosis of the tissue.

In a series of studies on household products and chemicals provided by the Colgate-Palmolive Research Center a good correlation was established between ocular irritancy and damage to the chorioallantoic membrane.

The tests cited above reflect overall irritancy. Hence, they are analogous to only a portion of the Draize test. Table 12 lists a variety of cell and organ culture systems which could be exploited for the evaluation of damage to different parts of the eye and different aspects of ocular function. Such tests could be used as screens. For example, a compound which is highly toxic to corneal cell function perhaps should not be tested in animals unless special needs justify further testing. Again - the greatest value of in vitro tests will be in decision making!

F. VALIDATION OF ALTERNATIVE SYSTEMS

Validation entails the systematic undertaking of experiments and retrospective analyses in order to demonstrate whether or not a specific test or experiment could be predictive of a similar event in another organism. Systematic validation is characteristic of toxicity testing; it is not done systematically in the development of research models. Most research models more or less surface from the scientific data which is reported for other purposes. An investigator may recognize that a

Table 12. - Proposed Components of Preliminary Test Battery to be Validated for Ocular Toxicity Testing

1. Corneal epithelium cell cultures from rabbit and/or humans. End-points: cell viability, cell proliferation, release of neurotropic factors, keratinization, multilayering.
2. Corneal endothelium cell cultures from rabbit. End-points: (Na^+ + K^+)- ATPase mediated pump. Synthesis of Descemet's membrane-like collagenous layer.
3. Isolated corneas from rabbit and/or humans. End-points: specular microscope evaluation of corneal thickness.
4. Lens epithelial cell cultures from chicken embryos and rabbits. End-points: cell viability; lens epithelial cell differentiation (elongaton and lens protein synthesis).
5. Cell of nonocular origin: human diploid cell line, WI-38, MRC-5, or IMR 90; mouse fibroblast established cell line (L929); human epithelial established cell line (HeLa). End-points: cell viability, cell proliferation, growth pattern.

useful model may emerge from various studies and then proceed to conduct in depth or superficial studies to validate the model. The model systems used in education settings are spin-offs of the research models. Hence, the focus of this section of the report is on validation of toxicity tests with an emphasis on screening tests because they offer the greatest promise as alternative systems.

Figure 2 shows the current and future relationships between in vitro and in vivo test systems (Nardone and Bradlaw, 1983).

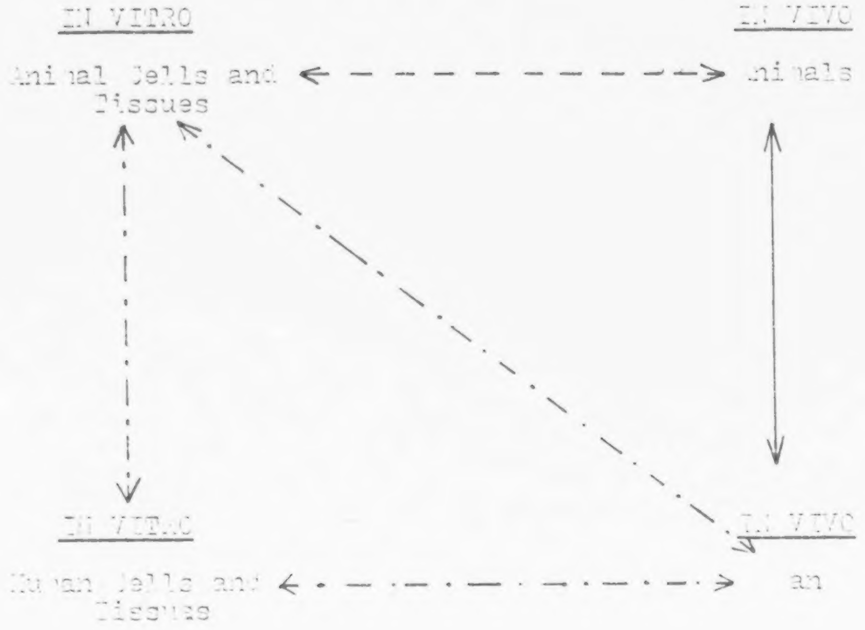
The established correlation of In Vivo Animals - In Vivo Man is the underpinning of animal toxicology aimed at predicting human risk. The In Vitro Animal Cells - In Vivo Animals correlation is where most of the current development work is being done. Should this development work prove to be productive, the extrapolation In Vitro Animal Cells - In Vivo Man will be possible in certain limited instances. In any event, the development work may significantly reduce the number of animals used for toxicology.

Nardone (1980) has commented on the potential use of in vitro systems in the following way.

> "Today we hear less about tissue culture as risk assessment and more about its value as a screening tool to assist in prioritizing limited resources and in other aspects of decision making. Instead of asking of in vitro studies, "What concentration of a xenobiotic will pose a risk of particular magnitude?" we are asking "Does a xenobiotic, toxic to cells in culture, pose a risk to man?" While this role may not appear to be grandiose as that of a surrogate for animal toxicology studies, its importance cannot be overestimated. The number of new products manufactured each year far exceeds our capacity for complete animal toxicological evaluation. This problem is compounded further by the output of industrial effluents and other complex mixtures which also require study. The in vitro two-dimensional microliter approach developed by Ekwall and Sandstrom for the study of combined toxicity by drug pairs is an example of an enhanced opportunity to look at combined effects quickly. Clearly, any meaningful screening which can lead to the control of potentially harmful products deserves serious and sustained attention."

Screening tests are designed to enable a large number of chemicals

FIGURE 2. CORRELATIONS BETWEEN *IN VIVO* AND *IN VITRO* TEST SYSTEMS


IN VITRO
Animal Cells and Tissues
IN VIVO
Animals
IN VITRO
Human Cells and Tissues
IN VIVO
Man

(——) Established correlations; (- - -) under development; (-·-·-) future considerations.

to be tested rapidly and inexpensively. They serve to provide some insight as to whether a chemical poses some risk to humans or other components of the bio-sphere. They are not intended to provide insights regarding the mechanism of action or the concentration or conditions which pose a special risk, although these may be spinoff benefits from screening studies. Screening studies should be viewed primarily as aides in decision making. They may alert concerned individuals to apply special handling procedures in the absence of animal toxicity data; they may suggest that a particular product be given a higher or a lower priority in development by a company; they may suggest which chemicals should be tested in animals and which should be abandoned without further testing; and they may lead to ranking the toxicity of related chemicals and suggest narrower dose ranges to be tested in animals. Ideally, screening tests yield unequivocal answers, but in reality false positives (nontoxic chemicals in animals causing a toxic response in the *in vitro* screen) and false negatives (a toxic chemical *in vivo* not causing a toxic response in the *in vitro* screen) arise. A good *in vitro* screen should have no false negatives. One could reasonably argue that when an *in vitro* screen produces no false negatives and when the screen yields data regarding the exposure (dose, route, etc.) which can be tolerated by man and animals with no attendant risk, animal tests will no longer be required. However, for the reasons cited earlier (metabolism, repair, complexity) this is an unrealistic aspiration.

Figure 3 is a schematic representation of the use of screening tests in decision making. Such decisions conserve resources, animals, and time because blatantly toxic chemicals or slightly toxic ones of little social or commercial value are discarded without further animal testing. Animal testing is reserved for those chemicals which are not manifestly toxic and which have significant value.

The prioritization capability which stems from the judicious use of cell culture screening tests has as a significant application the preliminary identification of chemicals already in use but which have not been tested for toxicity. The importance is illustrated by the following data reported by Griesemer (1981). About 4.5 million chemicals are known to exist. Only 6,000 of these have been tested in animals. New chemicals are being produced at a rate of about 700-3,000 per year but our worldwide resources for animal testing can handle no more than 300-500 per year. Furthermore, a back-log of 60,000 chemicals

FIGURE 3. DECISION TREES FOR THE TESTING AND HANDLING OF CHEMICALS INTENDED FOR LOW CONSUMPTION AND FOR HIGH CONSUMPTION.

CHEMICAL INTENDED FOR LOW CONSUMPTION OR RESTRICTED USE

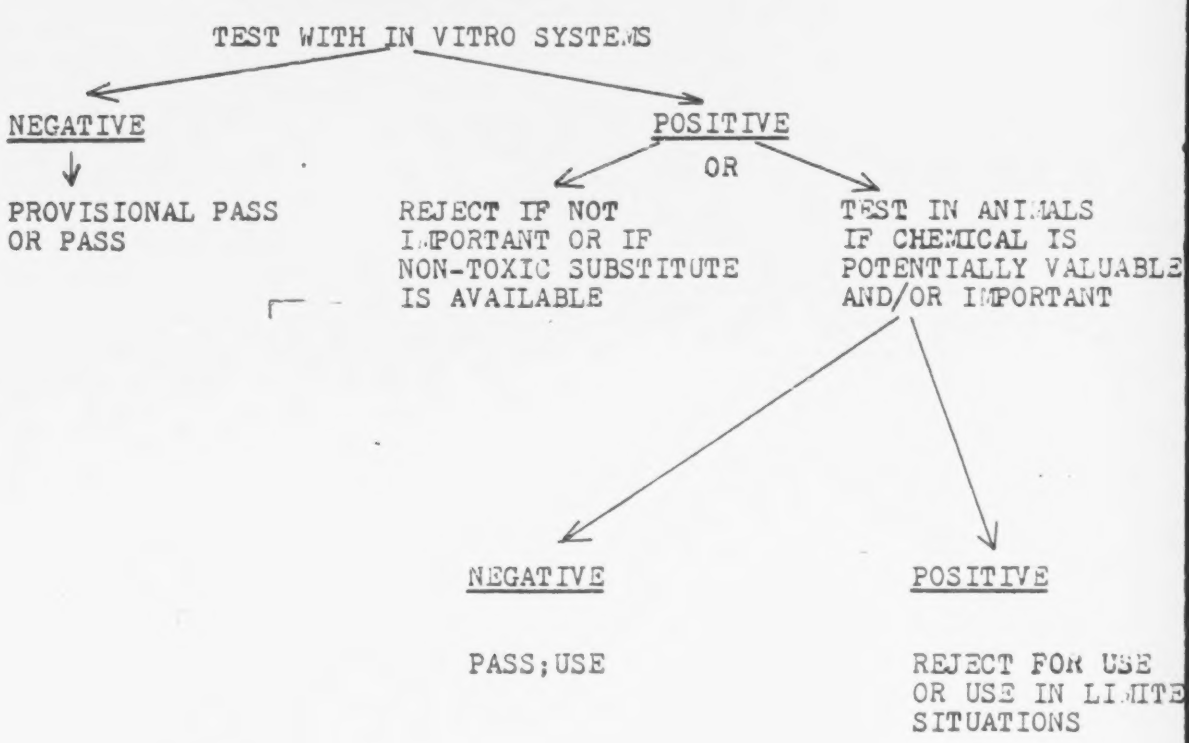
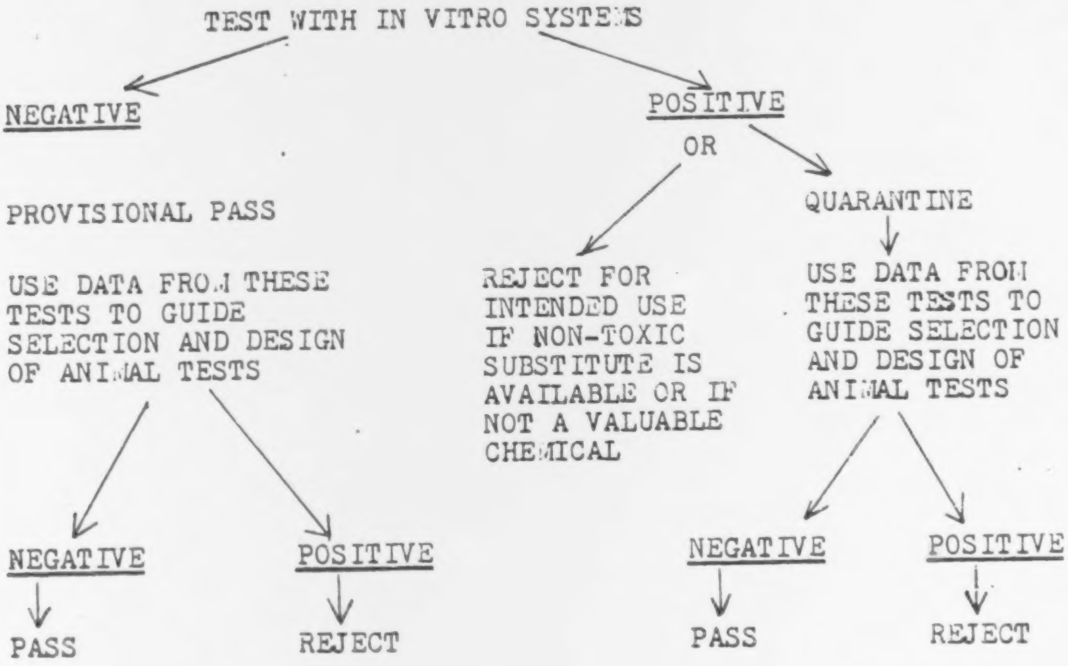

TEST WITH IN VITRO SYSTEMS
NEGATIVE
PROVISIONAL PASS OR PASS
POSITIVE
OR
REJECT IF NOT IMPORTANT OR IF NON-TOXIC SUBSTITUTE IS AVAILABLE
TEST IN ANIMALS IF CHEMICAL IS POTENTIALLY VALUABLE AND/OR IMPORTANT
NEGATIVE
PASS; USE
POSITIVE
REJECT FOR USE OR USE IN LIMITE SITUATIONS

FIGURE 3 CONT'D

CHEMICAL INTENDED FOR HIGH CONSUMPTION; LOW EXCRETION AND/OR DEGRADATION RATES



TEST WITH IN VITRO SYSTEMS

NEGATIVE

PROVISIONAL PASS

USE DATA FROM THESE
TESTS TO GUIDE
SELECTION AND DESIGN
OF ANIMAL TESTS

NEGATIVE

PASS

POSITIVE

REJECT

POSITIVE

OR

REJECT FOR
INTENDED USE
IF NON-TOXIC
SUBSTITUTE IS
AVAILABLE OR IF
NOT A VALUABLE
CHEMICAL

QUARANTINE

USE DATA FROM
THESE TESTS TO
GUIDE SELECTION
AND DESIGN OF
ANIMAL TESTS

NEGATIVE

PASS

POSITIVE

REJECT

in production have yet to be tested in animals (Schreiner, 1983).

The in vitro studies, if they are to be useful, must be predictive of in vivo effects. In order to determine whether or not such predictability is forthcoming a series of validation experiments are carried out. Ideally, the in vitro and in vivo experiments are conducted simultaneously. However, in practice, the in vitro studies are based on retrospective analyses of in vivo toxicology reports. For example, in such a study on the usefulness of cultures of neuroblastoma cells to predict the potential neurotoxicity of neurotoxic organophosphates, Fedalei and Nardone (1983) ranked the effect of six known animal neurotoxins. They reported that the toxicity ranking for the inhibition of neurotoxic esterase in vitro was similar to the in vivo ranking. Furthermore, they demonstrated that the toxicity was specific and not the result of general cellular toxicity.

Similarly, Acosta and Sorensen (1983) have shown that cultured hepatocytes could be used for the specific evaluation of hepatotoxicity and Tarr et al (1983) have shown a good correlation between in vivo and in vitro immunotoxicology, at least in a limited way.

It should be pointed out that these studies selected specific end-points for evaluation. However, the nervous system, the liver, and the immune system are multi-faceted and have many specialized activities. In order to use cell cultures, for example, to test for diverse chemicals capable of causing diverse toxic responses, a complex battery of tests must be used. Nardone (1983) and Nardone and Bradlaw (1983) have outlined such strategies for the development of test batteries for the evaluation of neurotoxicity and ocular toxicity, respectively. Table 11 summarizes the components of a proposed test battery for neurotoxicity which can be validated immediately with little further development work. Table 13 summarizes the steps required for such validaiton (Nardone and Bradlaw, 1983). This validation procedure incorporates some of the recommendations stemming from the working conference of The Tissue Culture Association Committee on Toxicity Testing held in 1975 (Berky and Sherrod, 1977).

Although the test battery described in Table 8 involves a number of end-points, it reflects only a small fragment of neuronal function. Nevertheless, such a limited battery could be useful for screening purposes. Chemicals which severely and adversely affect any one of the end-points listed therein may not justify further testing. Its toxicity would have been demonstrated. Compounds which clear the screen (proved to be non-toxic) with respect to the end-points listed therein, would be monitored with animal or other tests before being declared safe. From a humane point of view, this would reduce the number of animals used and would reduce the level of insult they experience.

In order to better appreciate the underlying rationale for alternative test battery development, let us analyze the proposed test battery for neurotoxicity testing (Table 8).

The model put forth in Table 11 entails studies with four different biological systems with overlapping characteristics. The mouse neuroblastoma cell line NIE-115 is a transformed (tumor) cell which displays several of the neuronal properties listed in Table 11 which will serve at test end-points. The cell is immortal in tissue culture and has the added attribute of behaving like an unspecialized cell under some culture conditions and like a nerve cell under other conditions. Hence, the same cell can be used for the evaluation of toxins on general cell function and on some aspects of neural function. The hybrid cell line NG108-15 has many of these attributes. Hence, it can serve as a basis for comparing two different cell lines. It has the added attribute of establishing synapses with muscle cells in culture. Hence, should the test battery warrant acceptance and expansion, a very important end-point, - the ability to transmit a nerve impulse from nerve to muscle - could be added. The chick brain cell aggregate system and cortex organ culture are primary cultures established from chick embryo brain. The aggregates permit the evaluatjion of cellular toxicity in a three-dimensional matrix and some characteristics of brain development. The brain organ culture permits the evaluation of different cellular components of the brain while maintaining the normal architecture. The mouse primary spinal cord culture is included to provide a basis for comparing the toxic response of the established, continuous cell lines (NIE-115 and NG108-15) with cells just removed from an animal. The human cell line is not of neuronal origin. It provides a basis for comparing neuronal cells with non-neuronal cells. The neuronal end-points selected for inclusion in the test battery reflect important activities of the function and development of the nervous system. The list, however, is only a fraction of the list which could be generated and which may be included in a second generation test battery.

The non-neuronal end-points are important ones for the evaluation

Table 13. - Studies Required for Test Battery Validation.

1. A variety of toxicants, whose mechanisms of toxicity and whose *in vivo* toxicity rankings are known, should be used. These should include those that affect the diverse end-points listed in Table 8.
2. Dose-response studies, to be used for *in vivo* ranking, should include the establishment of the minimal toxic dose, the maximal toxic dose, and the determination of the slope of the inhibition curve.
3. Negative controls as well as positive controls should be used.
4. The toxicants should include representatives of many chemical classes.
5. The doses used *in vitro* should include those which approximate those which exist at the point of cellular contact *in vivo*.
6. The data should be analyzed to determine the frequency of false positives, false negatives, and ambiguous results.

of injury to any proliferating cell. They are included here in order to determine whether or not the toxicity of chemicals preferentially affects neuronal or non-neuronal end-points.

G. ADVANTAGES OF USING ALTERNATIVE METHODS

The concept of alternatives can take on many different meanings depending on which group is using the term. For example, one group may be referring only to those techniques which lead to total replacement of laboratory animal use while another will include a broader range such as techniques which reduce animal pain and suffering. For the purpose of this discussion, the term alternatives will be used to refer to the three "Rs" defined by Rowan (Mice Models and Men, 1984), which are: Replace the use of laboratory animals altogether; Reduce the number of animals required; or Refine an existing procedure or technique so as to minimize the level of stress endured by the animal.

When one considers the use of alternatives in these terms, several advantages become obvious immediately. First, replacing or reducing the number of animals used would immediately save the investigator time, money and space. Time because several in vitro methods that have been developed and described earlier can be performed more quickly. For example, carcinogenesis testing in a rodent is a study that can take as long as 18 months whereas in vitro cell transformation can be performed in one month. This time saving advantage leads to an added benefit, namely, the ability to perform the experiment repeatedly thereby permitting confirmation of the test results in less time than it would take to do the initial experiment once using live animals. Secondly, is the added savings in experimental dollars and space. Government regulations that have resulted from the concern expressed by animal welfare groups, has made the use of animals a costly endeavor. Laboratories that utilize animals for research must have an on-site veterinarian or have one visit the site regularly. Housing and maintenance of the animals can also be a very costly endeavor. These costs are further increased because animal experiments take months to perform.

In addition to the above mentioned advantages of using alternatives, there are several other, more subtle, reasons for devoting time and money to the development of alternatives. First, some experimental methods are far more sensitive and powerful than the same study done in animals. Before the advent of chromatography (gas, liquid and HPLC) and mass spectroscopy, fat-soluble vitamins were necessarily assayed in animals. However, these new biochemical techniques offer greater sensitivity and more assays can be done on smaller amounts of material than could be done in the past with the use of animals (Wiggins, 1976).

Microbiological systems have, in a few cases, totally replaced the use of animals as in the vitamin assays developed by Gyorgy and Pearson (1967) but more commonly they are employed to reduced the number of animals required by serving in preliminary or pilot studies which will determine if further testing is required as in the Ames test. The development of _in vitro_ cell culture techniques for mutagenicity, toxicity and carcinogenicity testing has brought with it many advantages to the investigator, not the least of which is the reduction in the use of animals. _In vitro_ studies have the added advantage of reducing experimental-induced artifacts due to the handling of animals which can have a profound effect on the animals, and the results obtained by their use. This is especially true of behavioral toxicology studies or studies which utilize nocturnal animals. _In vitro_ studies give the researcher greater flexibility to change the conditions and variables of any given experiment to broaden the scope of the study. An example of this is the closed _in vitro_ system developed by Endotronics which allows the investigator to control the environment of the cells very precisely by computer commands which control the flow of certain activators or suppressors which are released at precise time intervals. Such a completely controlled environment allows the investigator to note the most subtle changes because all the effluent can be analyzed and the data fed into the computer for quick, accurate results. Such a system also reduces the level of error which can result from interindividual variability. These types of errors are not easily detected but can nevertheless alter the results of the experiment. This is not always true even when using in vitro systems. For example, until recently, the cell lines being used for in vitro testing were in many cases heteroploid or transformed and the use of such cell lines can cause variation in a test system because of the instability of the cells themselves. More recently, researchers have developed systems which use normal human diploid cells rather than established cell lines (Thilly et al., 1977; Sato et al., 1972 and Thilly et al., 1976).

In some cases, tissue culture systems have replaced the need for animals entirely. A notable example of this is the use of cells for the virus vaccine production and some vaccine potency and safety tests (Petricciani et al., 1977). In general, however, the main potential of tissue culture systems lies in the ability to use the technique to reduce the demand for animal research. For example, a pharmaceutical virology laboratory reduced its yearly demand for mice from 13,000 to 1,600 between 1963 to 1975. At the same time, they increased the number of compounds screened yearly from 1,000 to 22,000. These changes were due entirely to the employment of cell and organ culture systems as preliminary screening systems before testing the most promising chemicals in the mouse system (Buchnall, 1980).

Another important advantage of using in vitro systems lies in the intrinsic potential they possess for a much broader spectrum of parameters. Because cells can be isolated and placed in a controlled environment, they lend themselves to test methods that can give quantitative as well as qualitative information. Examples of some of the parameters that can be studied using cells are cell viability, cell reproduction, macromolecular biosynthesis, differentiated function, and structural aberrations. Furthermore, each of these parameters offers the investigators several methods of evaluation. Case in point, cell viability can be evaluated in any one of the following ways: a) vital staining with an exclusion dye, b) colony formation for substrate-attached cultures, c) plating efficiency for substrate-attached cultures, d) colony formation by transformed cells in soft agar, and e) chromium 51 release. Similarly, cell reproduction can be studied from a kinetic aspect as well as by looking at the effect on population doubling or mitotic index. Macromolecular studies can determine the DNA, RNA and protein content in a cell, or examine the incorporation rates of their precursors as well as the fidelity of DNA transcription or the induction of DNA repair. Test methods related to differentiated function and structural aberrations enable investigators to look at cell surface-membrane responses, such as receptors. One might also look at membrane fluidity using electron spin resonance techniques. Organelle function can be determined by evaluating the mitochondria with respect to glycolysis, ATP and O_2 consumption. And finally levels of ATP, cyclic AMP and GMP and other pool sizes can be determined to see if the cells are behaving normally.

Finally, it is advantageous to use in vitro methods for testing the potential hazards of agents because a high positive correlation has been shown, in some studies, between potential carcinogenic activit in vivo and mutagenic activity in vitro. Comparative studies of this type are relatively scarce; however, one notable example is the work summarized by Lutz (Lutz, 1979). In order to evaluate whether mutagenicity data *in vitro* could also predict quantitatively the processes that occur in intact mammalian organisms, the microsome-mediated mutagenicity in *Salmonella* was compared with the covalent binding index (CBI) of chemicals in rat liver *in vivo*. Forty four compounds were tested in this fashion and eight of the chemicals that were observed to bind covalently to rat liver DNA *in vivo* did not produce microsome-mediated mutagenicity in *Salmonella* strains. Apart from these eight, all the remaining 36 chemicals showed a positive correlation between CBI and mutagenicity *in vitro* in a double logarithmic plot.

A similar study was performed to compare the *in vitro* and *in vivo* correlation of toxicity of thousands of biomaterials (Guess et al., 1965). In this report, the investigators determined *in vitro* toxicity of the compounds by using an agar overlay on a monolayer of L-cells and compared the toxicity to the cells with the cytotoxic response of the same chemical in an *in vivo* rabbit implant. In each case where a tissue response was observed in the rabbit, a cytotoxic response was seen in tissue culture. Since the tissue culture method was more sensitive than the muscle implant, there were occasions in which "nontoxic" biomaterials, as determined by the muscle implant showed a cytotoxic response in the cells. There were no reported instances in which a non-cytotoxic biomaterial tested in the tissue culture method showed a positive or tissue response in the rabbit muscle. The cell culture method, therefore, seems to be an excellent screening method that is also very reliable.

Similarly, with the advent of mammalian hepatic metabolic activation systems (Ames, 1973a) several studies followed to show the correlation between carcinogens and mutagens. McCann et al. published the results of a carcinogen correlation study with 300 chemicals in 1976 (McCann and Ames, 1976). This analysis stimulated other similar "validation" studies to be performed over the next eight years. The findings, specifically the extent of correlation between carcinogens and mutagens, fell into a broad range of results. However, these studies compared the Ames test results with the effects of the same chemicals on rodents. At times, different species respond differently than humans and consequently the even greater need for the development of in vitro test systems that will be reliable indicators of what will happen in man.

In 1980, the U.S. Environmental Protection Agency sponsored a Genetic Toxicology (Gene-Tox) conference on the current status of bioassays in Genetic Toxicology. At that time, several prominent investigators in the field were brought together for round table discussions to assess the progress that had been made in the field of genetic toxicology and try to update the information available on tested chemicals listed in the Gene-Tox data base. These types of scientific exchanges of information are invaluable because they enable scientists to have a reliable source to reference for up-to-date facts related to a particular field of endeavor. Perhaps if all the information available on drugs, chemicals, compounds and the like that have been evaluated from a toxicological or mutagenic aspect, were assimilated in one place, the data generated might provide the information necessary to develop more reliable tests.

H. Disadvantages of Using Alternatives

Overall assessment of the progress that has been made to improve, refine and develop new techniques for use as animal alternatives indicates that the use of alternatives is not without its attendent problems and disadvantages. The major drawback associated with an attempt to completely eliminate the use of animals in research, besides being naive and unrealistic, is the handicap it places on probing the unknown in basic research studies. The term biomedical research is used to describe a wide variety of activities. However, basic research is very different from toxicity testing and the evaluation of agents in terms of safety, in both approach and goals. Furthermore, screening substances for toxicity or mutagenicity is very different from risk assessment to man. Basic biomedical research attempts to build up a composite picture of the manner in which a living entity functions and a great deal of time is spent testing theories to advance our basic knowledge and understanding of these physiological concepts.

In addition to the vital need and use of animals in basic research, biomedical science encompasses the use of animals in a number of other ways such as: a) for the extraction of products, b) for the production of biologicals , c) in the diagnosis of disease, and d) for biomedical education and the search for new drugs (Rowan, 1984). By the time alternatives for the above uses of animals are developed, there probably will be new and diverse reasons for their use and the alternatives will provide the time, space and funds necessary for such research.

Another major limitation of alternatives is the inability to study growth processes and the interaction of cells, tissues and organ systems due to the very limited and specialized nature of the _in vitro_ systems. By moving further away from the intact animal system, it becomes increasingly difficult to elicit normal biological responses from the tissue (Gruenberg and Peluso, 1983). This is due to the inability of _in vitro_ systems to effectively simulate the dynamic aspects of _in vivo_ fluctuating regulatory factors, such as hormones, electrolytes and metabolites. Perhaps in the future, a continuous-flow tissue culture process will be developed which will enable the delivery of various substances to isolated tissues in a manner which emulates known _in vivo_ patterns, but current technologies do not provide such tools. Furthermore, it is impossible to study development processes and all of the complex interrelated mechanisms involved in the growth and maturation of an animal. There is no *in vitro* test or collection of *in vitro* tests that can accurately reproduce the enormous complexity of the living animal. Consequently, if one is to determine the effect of an agent as a potential mutagen, one must be able to be sure that the active form of the chemical is able to reach the germinal tissue. This is crucial in assessing the potential of a mutagen to cause heritable germline mutations and should be considered in assessing risk to humans, along with considerations of its carcinogenic potential (Hollstein et al., 1979).

Finally, animal alternatives do not enable the investigator to study biochemical and metabolic pathways nor do they permit studies which differentiate between male and female or species-specific responses. For example, differences between the effect of a toxin between males and females could be under direct endocrine control. Therefore, the experimental protocol for both acute and chronic toxicity tests generally specifies that the tests include both sexes. One of the most striking examples of a male-female difference in toxicity is the nephrotoxic effect of chloroform in male mice of certain strains. Female mice of the same strains show virtually no effect from chloroform exposures that are lethal for the males (Casarett and Doull, 1975).

In conclusion therefore, it is important to increase the level of awareness and understanding within the scientific community, if alternative methods are to be expanded and utilized. Then and only then can change be effectively accepted and implemented. Individual scientists, professional societies, research institutions and animal welfare groups must all work together toward a common goal that will satisfy the overall needs of the diverse entities involved as well as society.

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OVERVIEW OF COMPUTER USE IN
RESEARCH, TESTING AND EDUCATION

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TABLE OF CONTENTS

Page

1. Task 1: A Centralized Registry For Existing Biological Experimental and Test Results

I. Introduction

II. Premise

III. Discussion

2. IV. Exchange of Information in the Research and Testing Communities

4. V. Anticipated Benefits from a Computerized Registry of Baseline Biological Data

A. Decreases in use of animals in testing and research

6. B. Checking for genetic drift

C. Providing new ideas for research

7. D. Prevention of unintentional duplication of animal testing and experimentation

8. VI. Laboratory Animal Data Bank (LADB)

A. Origins of LADB

B. Building phase

9. C. Public accessibility

11. D. Reasons for failure of LADB to survive

12. IV. Lessons Learned from LADB

A. Recommendations of the FASEB LADB User Assessment Panel

13. V. Expansion of the LADB Concept to Include All Vertebrates

VI. Maintenance of a Centralized Biological Data Registry for Baseline Data

A. Sources of data

14. B. User community for LADB

C. Responsibility for maintenance

19. Task 2: Computer Modeling of Biological Systems

I. Introduction

II. Use of Computers in Cellular and Subcellular Biological Processes

20. III. Single Organ System Models

21. IV. Use of Models in Education

23. V. The Uses and Availability of Computer Models in Biology

25. Task 3: Computerized Predictive Structure-Activity Evaluation

I. Basic Concepts of Structure-Activity Relationships

A. Definition of SAR

B. Representations of "Structure"

26. C. Representations of "Activity"

27. D. Relationships between "Structure" and "Activity"

29. II. Development of Mathematical Models of Toxic Effects of Chemicals

A. Rat Oral LD_{50} models

30. B. Carcinogenicity models

32. C. Teratogenesis model

35. D. Mutagenesis models

E. Draize test models

37. III. Current Status of Utility and Limitations of These Models

41. Task 4: Hardware Considerations

I. Discussion

43. II. Centralized Computer Registry of Biological Test Results

A. Mainframe computer

B. Terminal requirements

III. Attitudes of Research Funding Agencies Towards Computers

A. NIH

44. B. NSF

IV. Computer Requirements for Biological Modeling

A. SAAM and CONSAM (NIH Simulation and Modeling Programs)

45. V. Computer and Data Requirements for Mathematical Modeling of Toxicological Endpoints

A. Hardware considerations

B. Software considerations

C. Availability and cost considerations

46. D. Data considerations

47. References

Task I: A Centralized Registry for Existing Biological Experimental and Test Results

I. Introduction

The information resources available to the scientific community are many and varied. These will be briefly described, and a gap in the coverage of animal baseline (or control) data, which first became apparent in 1973, will be described, together with an unsuccessful attempt to meet that need. The reasons for this initial failure will be analyzed, and recommendations made by an independent group to improve on that effort will be discussed. Possible advantages which would be realized by such a system are identified.

II. Premise

For purposes of this report it is important to distinguish between "biological experimentation" and "biological testing." The use of living organisms as research subjects for experimentation is basic to biomedical research, whereby new understanding is obtained, or refuted. The repetitive use of a standard biological test situation, (or protocol), employing different chemicals or differing test parameters, is "biological testing". Both experimentation and testing may involve whole animals, or organs or tissues taken from living organisms. Biological testing is usually concerned with determining the toxicity (or safety) of chemical substances.

III. Discussion

A centralized data base (necessarily computerized) containing evaluated data concerning biological baseline (or control) data for specific strains of animals should reduce use of animals in experimentation in several ways. First, by permitting selection of an appropriate animal strain, based on experimental data already obtained, but usually not easily retrieved from the scientific literature. Second, by identifying the desired information itself, should it already have been determined, thus avoiding unintentional duplication of animal experiments. Third, by aiding the efficient design of test protocols, thus minimizing the number of animals necessary to be tested to ensure statistically significant results. "Evaluated" data means that the data have been reviewed and approved by a group of experienced biological scientists - the "peer review" process.

Experience gained in a recently terminated effort to develop such a data registry for control data of a few mammalian species (The Laboratory Animal Data Bank, "LADB") strongly indicates that expansion of the scope to include all research experimental and test results on all vertebrate animals would increase the complexity, size and cost of such a data base at least 100 fold, which is far beyond any reasonable magnitude. Thus, increasing the scope from rats and mice to all higher vertebrates would result in at least a 5 fold increase in these factors, and the inclusion of all research data from experiments on vertebrates would add at least another 20 fold increase. The existing biological experimental and testing publication services (abstracting and indexing services of BioSciences Information Services and of Chemical Abstracts Service) provide access to the entire biomedical published literature, although not at the detailed level which set LADB apart from these traditional resources. The production of these abstracts, which permit limited retrieval of all experimental and testing studies on all species, costs more than $25 M per year, which comes from the private sector. The LADB activity was funded by the federal government for slightly more than $3M total from 1975-1980, and included in-depth baseline data for rats and mice, and some data for other mammals.

For this report, a "Centralized Biological Registry" will be limited to baseline (control) data for mammals.

IV. Exchange of Information in the Research and Testing Communities.

The basic commodity in science is information. Science could not advance beyond the capabilities of one or a few individuals were it not for the ability to build upon the experience of others, both ones' predecessors and contemporaries. Publication of scientific knowledge, observations and discoveries, can be made in scientific journals (primary literature); access to these publications is provided by abstract journals and their indexes (secondary literature); and various compilations, such as books, monographs and reviews, constitute the tertiary literature. Most primary publications require review by ones' peers, prior to acceptance of articles for publication. In the fields of biology, several hundreds of thousands of scientific articles are published each year in the primary literature. Most of the major fields of biology are well served by scientific societies, whose major functions include publications and scientific meetings, at which the latest scientific advances are presented. Some organizations also prepare abstracts (secondary literature) and indexes thereto, in order to make it easier for scientists to monitor the voluminous literature for relevant publications. Indexing is made by subject terms, by author, and by citations (references). Citation indexing permits one to follow the literature into the future, and provides an alternative approach to the use of abstracts and indexes for identifying pertinent articles.

All of these mechanisms serve the basic biological research community's information needs quite well. However, the biological testing community is more poorly served, for several reasons. First, the results of testing for adverse biological effects often yield negative results, and these are traditionally difficult to interest the basic scientists who usually comprise the peer reviewers for publications. Hence, most negative tests go unreported, and may be repeated in the future for the lack of documentation. Second, many test results are obtained by researchers in the pharmaceutical, pesticide or chemical industries. These results may frequently be needed for regulatory purposes. These are usually considered as proprietary data, publication of which might aid one's competitors. The data on testing of control (untreated) animals (which may not be considered to be proprietary) are rarely published by these industries, because they are not considered to report new scientific knowledge.

The National Library of Medicine made publically available in 1978 a special information service, the Toxicology Data Bank (TDB), designed to address some of the needs of the testing and regulatory communities for toxicity information. TDB is not document-oriented, but is organized by individual chemicals or substances which now total more than 4,000 in number. It employs a fixed format which includes data on the production and use of a chemical, its physical properties, and especially, the results of pharmacological and biochemical experiments and toxicological testing. However, because it uses conventional literature sources, it does not include baseline data on control animals, which are rarely published. TDB's main strength is in its peer review of all data, which make its summaries of biological data acceptable to most users. The very same process (peer review) necessarily introduces long time lags for the introduction of new or revised data, and much of the data are from 1 to 2 years behind current developments.

The Registry of Toxic Effects of Chemical Substances (RTECS) has been published annually since 1971 by the National Institute for Occupational Health, under a Congressional mandate. RTECS is currently available both in hard copy form and on microfiche, and is available online from NLM and the NIH-EPA's CIS system. The production of RTECS requires about $500,000 each year. The latest quarterly update includes a total of 68,000 compounds, and it is estimated that it will grow to include some 100,000-150,000 compounds by 1995. If RTECS were permitted to include all results of in vivo toxicity testing (including negative results), its size would be increased by only an estimated 10 to 15% .

Several online data bases available from NLM are compared in Table 1.

Thus the research and testing communities have differing needs for information and for publication, although they both use scientific methods. This was the genesis of the original LADB effort. Such a data registry would be of major utility to the biological testing community, rather than to the basic biological research community.

V. Anticipated Benefits from a Computerized Registry of Baseline Biological Data.

A. Decrease in use of animals in testing and research

In some cases one would locate the exact data desired, (possibly from an unpublished source), thus avoiding unintentional duplication of animal testing.

SELECTED NLM ONLINE DATA BASES

Name	Brief Description	Number of Records (Ave. length)	Basic Type of Record
MEDLINE	1966-present. Bibliographic citations and abstracts from primary biomedical literature	3,300,000 (1,250 characters)	Bibliographic
TOXLINE	1965-present. Abstracts from primary toxicological literature	1,400,000 (1,050 characters)	Bibliographic
CHEMLINE	Dictionary to chemicals contained in TOXLINE	500,000 (275 characters)	Chemical Compound
RTECS	Brief summaries of toxicity resultsfrom primary literature	63,000 (about 1,000 characters)	Chemical Compound
TDB *	Detailed chemical, pharmacological and toxicological data and extracts from monographs and handbooks	4,000 (17,000 characters)	Chemical Compound

* Removed from NLM's Data Base Set, August, 1981

Table 1.

It would allow more efficient design of a proposed test, by providing baseline data to permit selection of a dose, a route, and a strain of test animal without the need for new animal experiments to establish these factors. Efficiencies should include the use of fewer doses on smaller numbers of test animals than would have been necessary without recourse to the registry.

The number of animals required for control groups could conceivably be reduced, but many biological tests require the use of concommitant controls, rather than of pooled control data, in order to achieve statistical significance.

B. Checking for Genetic Drift

The registry would provide baseline data within specified time frames of measurement. Certain data will change over a span of many generations due to "genetic drift". For example, over a period of some 15 years, the effective dose of chlorpromazine required to block the conditioned avoidance response in rats changed from 10 to 16 mg/kg, using the same strain of rat and the identical test protocol. (Smith Kline and French Labs). The use of a computerized registry would make it easy to check for the possibility of genetic drift, and could also lead to correlations between different types of genetic change.

C. Providing New Ideas for Research

By performing statistical comparisons across data sets, thereby identifying interrelationships not already obvious, one could establish unforeseen relationships without testing. (This cannot readily be done on the old LADB due to design deficiencies which would necessarily have to be corrected in a new registry.

A computerized registry does not offer much potential to reduce the use of animals in basic research, in contrast to testing, but the potential for seeking correlations between various sets of baseline data and new research data does promise to expedite the generation and testing of new research ideas.

D. Prevention of Unintentional Duplication of Animal Testing in Experimentation

It is accepted that some animal testing must necessarily be duplicated:

a). to provide scientific proof of new findings, reproducibility of test results must be possible. It is especially important to duplicate and thereby, verify, unexpected results which may not be in accord with accepted theory.

b). in education of medical personnel, hands-on experience with animals is essential for training of surgeons in working with tissues and organs. (Walker 1983)

c). in testing, use of positive controls is often required to ensure statistical significance of test results.

Unintentional duplication occurs when it is easier to test than to determine whether or not such a test was already carried out and reported in the scientific literature. The best sources for such information are the online versions of Biological Abstracts and Biosis Previews. However, the indexing is very broad, and one cannot always quickly locate the desired information. Most important, as long as only control (baseline) data are available in such a computerized registry, it will not be possible to avoid some unintentional duplication of animal experimentation in research.

Toxicity test results are often locatable in RTECS (the Registry of Toxic Effects of Chemical Substances), produced for NIOSH. However, by congressional mandate, only tests which indicate a toxic effect are to be included in RTECS; thus tests which show no toxicity are to be excluded. This can lead to repetition of toxicity testing of essentially non-toxic substances, which undoubtedly was not the intent of Congress. This is especially true for skin and eye irritation studies.

VI. Laboratory Animal Data Bank (LADB)

A. Origins of LADB

The Laboratory Animal Data Bank is a computerized set of records of baseline data of physiological, histological and other biological properties of mammalian species used in biomedical research. The Division of Cancer Cause and Prevention, National Cancer Institute, foresaw the need for better baseline data for experimental animals as the carcinogenesis bioassay program developed in 1970-1973. In 1973-4 the concepts which led to LADB were formulated by the Toxicology Information Program of the National Library of Medicine (NLM). The DHEW Committee for Coordination of Environmental and Related Programs (CCERP) provided sponsorship and funding for the design and development phases of LADB, with the National Cancer Institute acting as the major contributor.

B. Building Phase (1975-1980)

Battelle Columbus Laboratories was awarded a contract by NLM in 1975, after a competitive procurement, and began detailed design activities in 1975-6. Methods for obtaining data were developed, and the data file was designed to permit interactive access (time sharing) by users. Sufficient data were input to permit initial study by NLM staff in 1976, and a "Phase I" study was begun in 1977, allowing 13 outside users to test the system.

In June, 1976, NLM requested the Institute of Laboratory Animal Resources, National Academy of Sciences (ILAR), to provide advice on scientific and technical aspects of LADB. A Committee on Laboratory Animal Data was formed by ILAR to advise NLM. It met in 1977 and 1978 to consider reports from NLM and Battelle staff, and to respond to specific requests for guidance concerning LADB developmental aspects and Phase I feedback. NLM staff further requested ILAR to form a panel to review the basic concept, purpose, scope, validation of data and utility of LADB.

In 1978 ILAR prepared such a report, which included a recommendation that peer review of data for inclusion in LADB should be performed, together with peer examination of the criteria for data acceptability (ILAR, 1979). NLM contracted with the Life Sciences Research Office (LSRO) of the Federation of American Societies for Experimental Biology (FASEB) to organize an ad hoc LADB User Assessment Panel to review data descriptors and disciplinary coverage of LADB. In late 1979 each member of the LSRO ad hoc panel had a computer terminal with unlimited access to the LADB data base. The resulting "hands-on" experience provided the basis for an objective assessment of the data descriptors and the scope of coverage of LADB, which was reported in "Review of Data Standards Related to the Laboratory Animal Data Bank - Interim Report, March 1980, from LSRO-FASEB. This report recommends certain changes in the data elements, including addition of some new data elements. An additional 20 suggestions for improving LADB were also listed in this report (Altman and Fisher, 1980a). Table 2 lists the principal recommendations made in this report.

C. Public Accessibility

LADB first became available online to the public in April, 1980, via the Battelle computer in Columbus, Ohio.

Some 100 subscribing organizations logged a total of only 96 billable hours over the first six months of public availability. This usage was far lower than that for other data bases operated by NLM, even in their beginning months; and, coupled with the vigorous pressure to reduce long term federal financial commitments, the HEW Committee for Coordinating Environmental and Related Programs ceased funding the project in December, 1980. This led to the decision by NLM to stop LADB file building in January, 1981. Battelle continued to make the file available to the public until early 1982, but usage did not increase sufficiently to make it self-sustaining. The file was then turned over to the National Technical Information Service (NTIS) for public distribution via licensing. Slightly more than 1 million animal measurements were contained in the file when input was stopped. The majority of these were obtained from some 30,000 rats and mice. One copy of the LADB

SUMMARY OF MAJOR RECOMMENDATIONS
MADE BY LSRO PANEL AFTER REVIEW
OF LABORATORY ANIMAL DATA BANK*

1. Put the individual animal data file online with the animal group data file.
2. Standardize diagnostic terms for pathology data, by use of a system such as Systematized Nomenclature of Medicine.
3. Add new data elements to the LADB for growth, development, reproduction,and teratology.
4. Provide an online multivariate search capability for determining interrelationships between differing data sets.
5. Adopt new data acceptance criteria for data submitted to LADB.

* From Altman and Fisher, 1980

Table 2.

file has been licensed by NTIS to date, to Pergamon International Information Corporation, which announced in 1983 that it has joined with FASEB in a joint venture to publish hardcopy "Databooks" created from LADB records after review by FASEB. There are no plans to add data to the existing file, or to make it publicly available online.

D. Reasons for Failure of LADB to Survive

1. Financial Considerations

Although 34 online connect hours were logged by some 100 LADB users in December, 1980, only 96 hours total had been accumulated over the first six months of public availability. This was far too few connect hours to provide any useful base for self-sufficiency, which was one of the original goals for LADB. In the fall of 1980, the federal government was vigorously seeking ways to reduce long-term financial commitments. At this same time, NCI dropped its major financial support, and other DHHS agencies declined to pick up the slack under OMB pressures for reduced expenses. Hence the Battelle contract was terminated in early 1981 for lack of funding.

2. User Friendliness and User's Needs

In addition to financial problems, the interactive software (designed in 1975) predated many major software developments which have emphasized "user friendliness." The desired end user (biologist) thus found it hard to retain procedural familiarity with only infrequent use of LADB, which has special rules for its use.

Another problem with use of the data (identified in the first FASEB report, p 50) was the inability to perform online multivariate statistical comparisons between different data sets. This limitation, which makes some desirable comparisons difficult to perform, arose from inadequate design, and could be overcome if LADB were to be continued.

In the second and third FASEB reports (Dec., 1980 and June, 1981) recommendations are summarized which would ensure better definition of the user's needs. These are discussed in detail in the next section of this report.

3. Peer Review of LADB Design and of Data

Although ILAR (NAS-NRC) evaluated LADB in 1978, it had not been involved in original design considerations. FASEB entered the review process in 1979; again, it was too late for FASEB to have significant input into the design, or for most of the file building process. The March, 1980, FASEB review pointed out several major design problems, including lack of online availability of the individual animal data file (LADA), and the resulting inability to perform online multivariate statistical comparisons between different data sets. LADA permits searching for and correlating data obtained on each animal, but LADA is only accessible in batch mode (computer tape) and is not accessible online. LADB records, which are searchable online, are composite records from many animals; this prevents some desirable statistical correlations from being performed online.

IV. Lessons Learned from LADB

A. Recommendations of the FASEB LADB User Assessment Panel

In addition to the recommendations listed in Table 1, the FASEB LADB User Assessment Panel emphasized two major points required in planning and developing such a data base:

1. Clear definition of the potential users of such a system, and their specific needs which would be addressed thereby. No adequate study was performed by NLM or CCERP to clearly define the users and their needs, prior to the original design of LADB. The June, 1981, FASEB report, "Guidelines for Development of Biology Data Banks," outlines the need for such a study, and recommends certain steps which should be taken to ensure that a careful definition of need is obtained. The results of such a study should identify the various users, their needs, and their desire (or lack thereof)

to use and support such a data base, once it becomes available. (Altman and Fisher, 1981)

2. The critical need for peer review by experts, at all levels of development of such a data base. These levels include: system design, data element definition, establishment of standards, arranging for peer review of data during file building, and provision for feedback resulting from experience gained by actual use of the system (equivalent to evaluation of the system). The peer review process assures that many experienced scientists have agreed on the best design, standards and data to be used. The process ensures the best quality product, but inposes penalties of high costs and slow input of data.

V. Expansion of the LADB Concept to Include All Vertebrates

To ensure both the utility and acceptability of such a data bank, FASEB strongly recommends that its coverage be limited initially (for perhaps three years) to data for mice and rats as experimental animals, since these are by far the most frequently used species in both experimentation and testing. After rat and mouse baseline data are well covered, expansion to all other vertebrate species (including birds, fishes, reptiles and amphibians) would have to be carefully evaluated in order to ensure that such an increase in scope would not overload the data base. Since the bulk of animal experiments involve the use of mice or rats, the increase would probably be reasonable.

VI. Maintenance of a Centralized Biological Data Registry for Baseline Data

A. Sources of Data

It does not matter whether the baseline data are obtained from published or private sources, as long as they are evaluated to be of high quality by a peer review mechanism. The December, 1980, FASEB report shows that 44 organizations and 15 federal contractors had contributed data to LADB, and 9 other sources had agreed to do so when the collection of data was halted in January, 1981. A diverse set of private and public sources was included, and it appears that more good data was available than available funds permitted to be processed. (W. Hoag, Personal Communication).

B. User Community for LADB

The brief (six month) period during which LADB was publicly available was of insufficient duration to permit many conclusions to be drawn about the users. By definition of its content, the users would be expected to be pharmacologists/toxicologists concerned with toxicity testing, and especially, with chronic toxicity testing. Although this community is far smaller in number (about 3-5,000 scientists) than the basic biological scientist community (about 200,000 scientists) it is expected that the average toxicologist employs more research animals than does the basic research scientist. Hence the potential for a reduction in the number of test animals employed is higher for this baseline data bank than the 2 to 3% figure might suggest. This prospect was understood by the original designers of LADB to be one of the desirable benefits of the data bank.

C. Responsibility for Maintenance

1. No particular segment of the user population would make abnormally high usage of such a data registry; its value would reside in the infrequent but significant use by many scientists. Thus, only the federal government could be expected to finance such an undertaking for the common good.

2. The factors which led to the assignment of the LADB project to the National Library of Medicine, as a part of DHHS, would appear to be valid, should a similar project be desired in the future. NLM has related experience in handling substance-oriented bata bases (see Table L) such as the Toxicology Data Bank and RTECS (the Registry of Toxic Effects of Chemical Substances); the much larger NLM data bases, TOXLINE and the MEDLARS data bases, are bibliographic, rather than substance-oriented. The experience gained by NLM with the LADB project should ensure that peer review would

be done by expert biologists, especially those who are involved in chronic testing for endpoints such as carcinogenesis, teratogenesis and mutagenesis. The methodologies for such testing are undergoing steady development and improvement by basic research scientists, both in academia and in government, under major funding by NIH through its Division of Research Resources. However, the responsibility for testing is split between NIEHS, NIOSH, FDA, EPA, and CPSC, among others. Because of the lessons provided by LADB, it is essential that such a centralized registry should be under close control by peer review groups of scientists, including pathologists and toxicologists as well as basic research scientists. NLM has learned to rely on the peer review process for such specialized data banks as TDB and LADB; its experiences with LADB would ensure even more reliance at all stages by NLM on peer review for any related project.

3. Other candidate sources for operating such a centralized registry are listed below, with commentaries:

a.) The National Toxicology Program (NTP) - It did not continue the NCI major share of LADB funding when it combined with the NCI staff (in 1979-80) who were involved in the carcinogenesis bioassay program at NCI. NTP has never had as its mission the development of such a data base, and is not presently equipped to do so. Such a project appears to be out of scope for NTP's mission.

b.) The National Bureau of Standards , Department of Commerce, specializes in physical, chemical and engineering data bases, and has never been involved in a biological data base operation. It would not be a viable candidate.

c.) The Chemical Industry Institute of Toxicology (CIIT) lacks personnel experienced in large scale data base development or operation. Unless the chemical industry itself chose to increase funding to CIIT for this express purpose, it would not be a candidate for this activity.

d.) The Pharmaceutical Manufacturer's Association (PMA) is not independent of direction by its members (unlike CIIT in this respect) and also is not engaged in large data base efforts.

e.) The National Agricultural Library has not developed any specialized computerized biological data registry systems, so it would not appear to be a candidate source.

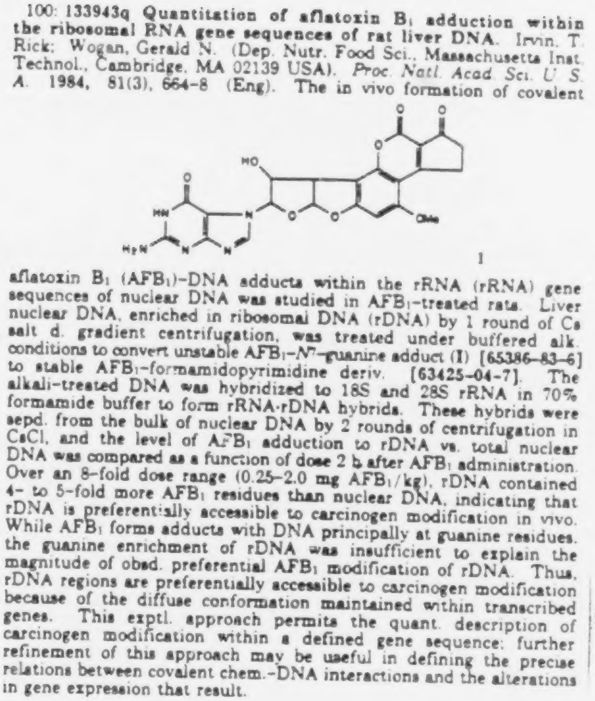
f.) Scientific organizations - these would appear to be viable candidates, based on experience:

1. The Chemical Abstracts Service of the American Chemical Society, and the BioSciences Information Services, both prepare hundreds of thousands of abstracts each year which report biological experimental and testing results. Samples are shown in Table 3. These files are document-oriented and indexed systematically, but not in the depth that LADB requires. (See Table 4) They could both take on such a task, and might consider doing so under a contract with NLM supervision.

2. The Federation of American Societies for Experimental Biology (FASEB) has published handbooks of biological data, and is currently embarked on a new venture to extract some data from the existing LADB data base. It might take on such a new project, provided adequate support was available. However, its role more properly would appear to be as the coordinator for peer review groups.

3. Because millions of dollars would be required over several years to design, develop, and maintain such a registry, it is readily apparent that no

A sample abstract from the Toxicology Section of Chemical Abstracts, 1984.



100: 133943q **Quantitation of aflatoxin B_1 adduction within the ribosomal RNA gene sequences of rat liver DNA.** Irvin, T. Rick; Wogan, Gerald N. (Dep. Nutr. Food Sci., Massachusetts Inst. Technol., Cambridge, MA 02139 USA). *Proc. Natl. Acad. Sci. U. S. A.* **1984,** 81(3), 664-8 (Eng). The in vivo formation of covalent

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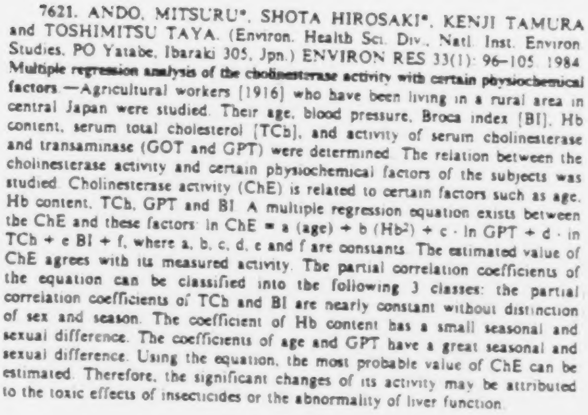
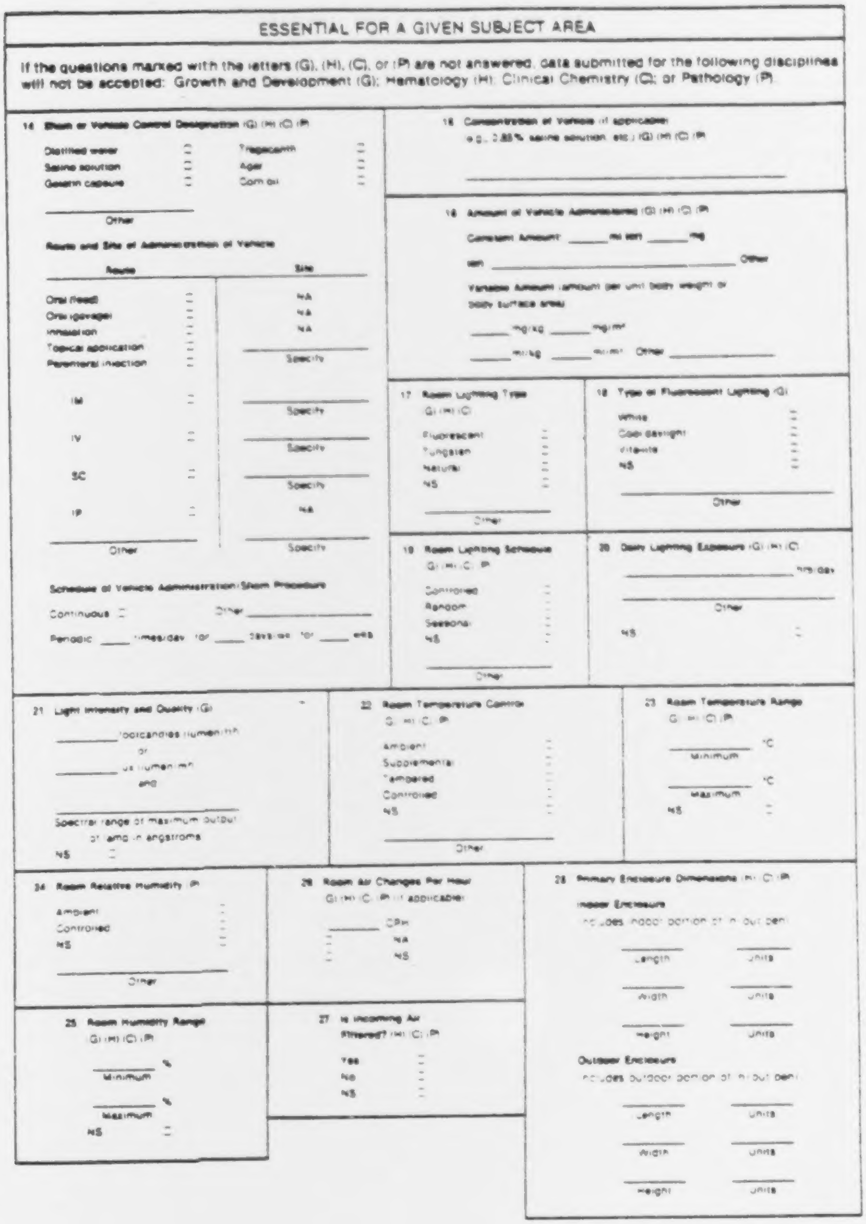
aflatoxin B_1 (AFB_1)-DNA adducts within the rRNA (rRNA) gene sequences of nuclear DNA was studied in AFB_1-treated rats. Liver nuclear DNA, enriched in ribosomal DNA (rDNA) by 1 round of Cs salt d. gradient centrifugation, was treated under buffered alk. conditions to convert unstable AFB_1-N^7-guanine adduct (I) [65386-83-6] to stable AFB_1-formamidopyrimidine deriv. [63425-04-7]. The alkali-treated DNA was hybridized to 18S and 28S rRNA in 70% formamide buffer to form rRNA·rDNA hybrids. These hybrids were sepd. from the bulk of nuclear DNA by 2 rounds of centrifugation in CsCl, and the level of AFB_1 adduction to rDNA vs. total nuclear DNA was compared as a function of dose 2 h after AFB_1 administration. Over an 8-fold dose range (0.25-2.0 mg AFB_1/kg), rDNA contained 4- to 5-fold more AFB_1 residues than nuclear DNA, indicating that rDNA is preferentially accessible to carcinogen modification in vivo. While AFB_1 forms adducts with DNA principally at guanine residues, the guanine enrichment of rDNA was insufficient to explain the magnitude of obsd. preferential AFB_1 modification of rDNA. Thus, rDNA regions are preferentially accessible to carcinogen modification because of the diffuse conformation maintained within transcribed genes. This exptl. approach permits the quant. description of carcinogen modification within a defined gene sequence; further refinement of this approach may be useful in defining the precise relations between covalent chem.-DNA interactions and the alterations in gene expression that result.

A sample abstract from the Toxicology Section of Biological Abstracts, 1984.



7621. ANDO, MITSURU*, SHOTA HIROSAKI*, KENJI TAMURA and TOSHIMITSU TAYA. (Environ. Health Sci. Div., Natl. Inst. Environ. Studies, PO Yatabe, Ibaraki 305, Jpn.) ENVIRON RES 33(1): 96-105. 1984. **Multiple regression analysis of the cholinesterase activity with certain physiochemical factors.**—Agricultural workers [1916] who have been living in a rural area in central Japan were studied. Their age, blood pressure, Broca index [BI], Hb content, serum total cholesterol [TCh], and activity of serum cholinesterase and transaminase (GOT and GPT) were determined. The relation between the cholinesterase activity and certain physiochemical factors of the subjects was studied. Cholinesterase activity (ChE) is related to certain factors such as age, Hb content, TCh, GPT and BI. A multiple regression equation exists between the ChE and these factors: $\ln ChE = a\,(age) + b\,(Hb^2) + c \cdot \ln GPT + d \cdot \ln TCh + e\,BI + f$, where a, b, c, d, e and f are constants. The estimated value of ChE agrees with its measured activity. The partial correlation coefficients of the equation can be classified into the following 3 classes: the partial correlation coefficients of TCh and BI are nearly constant without distinction of sex and season. The coefficient of Hb content has a small seasonal and sexual difference. The coefficients of age and GPT have a great seasonal and sexual difference. Using the equation, the most probable value of ChE can be estimated. Therefore, the significant changes of its activity may be attributed to the toxic effects of insecticides or the abnormality of liver function.

Table 3.



ESSENTIAL FOR A GIVEN SUBJECT AREA

If the questions marked with the letters (G), (H), (C), or (P) are not answered, data submitted for the following disciplines will not be accepted: Growth and Development (G); Hematology (H); Clinical Chemistry (C); or Pathology (P).

14 Sham or Vehicle Control Designation (G) (H) (C) (P)

Distilled water	☐	Tragacanth	☐
Saline solution	☐	Agar	☐
Gelatin capsule	☐	Corn oil	☐

____ Other

Route and Site of Administration of Vehicle

Route		Site
Oral (feed)	☐	NA
Oral (gavage)	☐	NA
Inhalation	☐	NA
Topical application	☐	____ Specify
Parenteral injection	☐	
IM	☐	____ Specify
IV	☐	____ Specify
SC	☐	____ Specify
IP	☐	NA
____ Other		____ Specify

Schedule of Vehicle Administration/Sham Procedure

Continuous ☐ Other ____

Periodic ____ times/day for ____ days/wk for ____ wks

15 Concentration of Vehicle (if applicable)
(e.g., 0.85% saline solution, etc.) (G) (H) (C) (P)

____

16 Amount of Vehicle Administered (G) (H) (C) (P)

Constant Amount: ____ ml with ____ mg

with ____ Other

Variable Amount (amount per unit body weight or body surface area)

____ mg/kg ____ mg/m²

____ ml/kg ____ ml/m² Other ____

17 Room Lighting Type
(G) (H) (C)

Fluorescent	☐
Tungsten	☐
Natural	☐
NS	☐

____ Other

18 Type of Fluorescent Lighting (G)

White	☐
Cool daylight	☐
Vitalite	☐
NS	☐

____ Other

19 Room Lighting Schedule
(G) (H) (C) (P)

Controlled	☐
Random	☐
Seasonal	☐
NS	☐

____ Other

20 Daily Lighting Exposure (G) (H) (C)

____ hrs/day

____ Other

NS ☐

21 Light Intensity and Quality (G)

____ footcandles (lumen/ft²)
or
____ lx (lumen/m²)
and
____
Spectral range of maximum output of lamp in angstroms

NS ☐

22 Room Temperature Control
(G) (H) (C) (P)

Ambient	☐
Supplemental	☐
Tempered	☐
Controlled	☐
NS	☐

____ Other

23 Room Temperature Range
(G) (H) (C) (P)

____ °C Minimum

____ °C Maximum

NS ☐

24 Room Relative Humidity (P)

Ambient	☐
Controlled	☐
NS	☐

____ Other

25 Room Humidity Range
(G) (H) (C) (P)

____ % Minimum

____ % Maximum

NS ☐

26 Room Air Changes Per Hour
(G) (H) (C) (P) (if applicable)

____ CPH
☐ NA
☐ NS

27 Is Incoming Air Filtered? (H) (C) (P)

Yes	☐
No	☐
NS	☐

28 Primary Enclosure Dimensions (H) (C) (P)

Indoor Enclosure
(includes indoor portion of in/out pen)

____ Length	____ Units
____ Width	____ Units
____ Height	____ Units

Outdoor Enclosure
(includes outdoor portion of in/out pen)

____ Length	____ Units
____ Width	____ Units
____ Height	____ Units

One of twelve code sheets required for input of pathology data to LADB

Table 4.

private information corporation with experience in large scale data base development would invest its own funds in such an operation. NLM would probably rely on contractors from the private or not-for-profit sectors, should it be assigned such a task.

Task 2: Computer Modeling of Biological Systems

I. Introduction

Modern approaches to physiology describe the functions of living systems at all organizational levels by the language of science--mathematics. Knowledge is acquired by investigating relationships among cells, tissues, fluids, organs, and organ systems. By trial and error, hypothesis, and testing, relationships begin to be understood, and can be described by mathematic expressions. These can range from simple linear functions, to various types of curved functions, to multidimensional surface functions, and may involve kinetic data expressed by differential equations. Parameters in these equations include physical terms such as time, temperature, weight, energy, force, volume, and motion. Complex mathematical relationships may be developed to better express these cause/effect relationships. In some instances a relatively simple relationship may be shown to exist, but this is unusual, because living systems are highly interactive and multidimensional in nature.

When such a relationship can reasonably be expressed in a mathematical equation, it may be considered to be a candidate biological model. The limits within which the expression will hold will determine the validity and utility of the model. If it is possible to change one (or more) parameters in the equation and thereby obtain the same response (s) found in animal testing, the model may be used to "simulate" a biological test or preparation. "Simulation" implies that the scientist can manipulate the parameters at will, and observe the resultant effects on the model. Simulation can provide useful teaching experiences, and is the basis for many educational models of biological systems. Simulation is also a useful tool for research and can suggest new mechanisms or hypotheses for further study.

II. Use of Computers in Cellular and Subcellular Level Biological Processes.

At the subcellular level, information is mostly gained by electron microscopic examination and analytical methods for sequencing of amino acids and nucleic acids. Such information tends to be of a descriptive and topological nature, rather than numerical. The exciting developments in genetic engineering which have resulted from increased knowledge of RNA, DNA, and protein amino acid sequencing and interrelationships, have required the computer to store and match nucleic acid and amino acid sequencing numbering in the millions of observations. (Orcutt, et al, 1983) These capabilities are not equivalent to modeling, but share with modeling in their joint reliance upon computers for storage and processing.

At the level of one or a few cells, models are being sought for computer simulation of sliding filament systems, which are believed to be basic to movement of flagella, cilia and muscle fibers. (Brokaw, Cal. Inst. of Tech., 1982). Modeling of the function of individual cone cells in the eye is under study at NIH (Pottala, et al, 1982).

However, most of the efforts towards modeling of biological systems are directed at higher levels of organization, such as organs, and organ systems. This is due to the complexity of living systems and the stabilizing influence of numerous feedback mechanisms, which are the basis for homeostasis. Many of these feedback mechanisms are located in several distant organs, and the entire system must be considered as a whole in order for the simulation to succeed. A good example is the need to consider the heart, brain, lungs and kidneys in a model of the cardiovascular system.

III. Single Organ System Models

Models are under development at NIH (Mejia, R., 1982) to describe the transport of electrolytes, non-electrolytes and water into and out of the kidney. Similar models are in use for instruction of basic renal physiology at Albert Einstein College of Medicine (Levine, 1984), at Clemson University and The Medical College of Wisconsin (Sias, et al, 1982).

Models of cardiovascular physiology are in use at Washington State

University (WSU) for instruction of medical, veterinary, and graduate physiology students. (Ringo, et al, 1982). The most recent development at WSU in teaching cardiac mechanics involves computer graphics, and displays a heart, permits the student to adjust at will the heart rate, the ionotropic state, the filling pressure and the afterload. The results of these changes are shown in graphic form (See Fig. 1) (Peterson and Campell, 1984).

At the basic research level, models of cardiac metabolism are under study at the University of Pennsylvania (Waser, et al, 1983) and at NIH a model is under study which describes cardiac pressure-flow-volume relationships (Moses, 1982).

Respiratory mechanics are being simulated by use of analog computer methodology at California State University, Fullerton (Khoo and Yamashiro), and at the Univ. of Mississippi. (Moffatt, et al, 1982).

The peripheral auditory system is being modeled at the University of Wisconsin, Madison; and single auditory nerve-fiber transmission of vibrations are under study. (Schwid and Geisler, 1982).

This is an incomplete listing, but it should serve to indicate the variety of organ systems under study, and the number and geographic distribution of scientists who are applying modeling techniques in their studies.

IV. Use of Models in Education

The use of models in the education of medical and graduate biomedical students is a relatively recent development, which has great potential for speeding their education at lower costs, due to the reduction in use of animals.

The savings result from several factors. First, use of a satisfactory model is far less expensive than the expenses incurred in obtaining and housing animal subjects. Second, a model is instantly ready for simulation, whereas an animal preparation may require from several minutes to several hours to be ready for study. An inadvertant overdose of a highly active chemical may kill an animal after a time-comsuming preparation; but in a

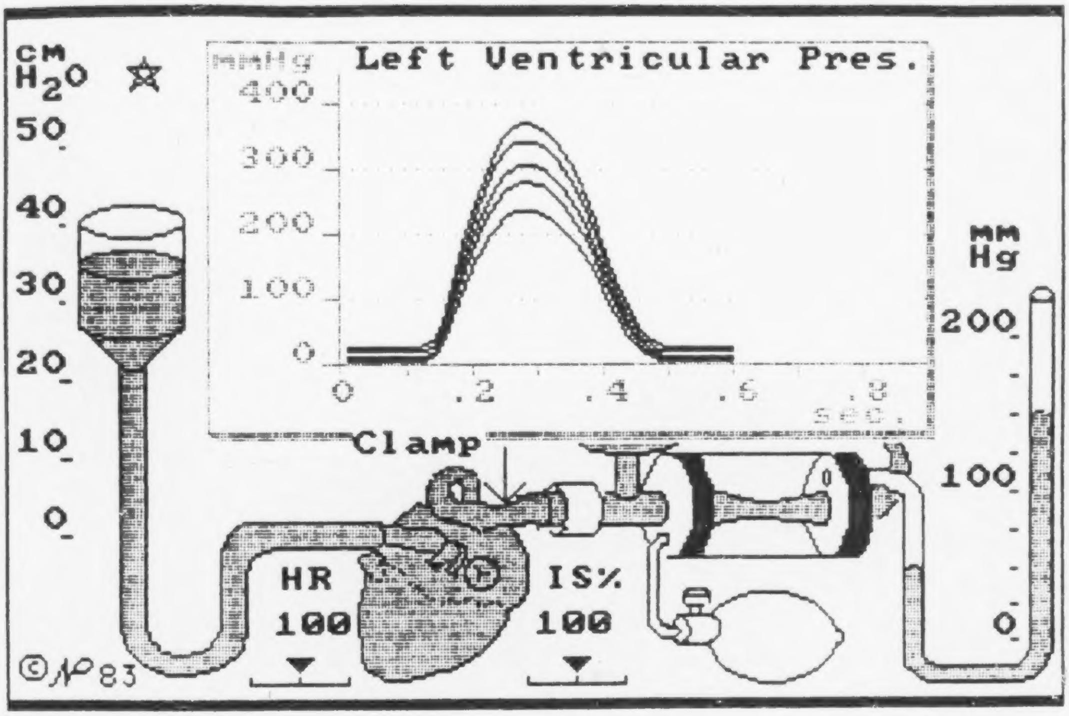

cm
H_2O
50
40
30
20
10
0
mmHg
Left Ventricular Pres.
400
300
200
100
0
0
.2
.4
.6
.8
sec.
Clamp
HR
100
IS%
100
mm
Hg
200
100
0
©№83

Figure 1.

model, one merely resets the simulation and starts over again. Third, certain animal preparations are so complex that they may not always seem to function according to the textbook; the model will respond as it should, everytime. (Walker, 1983).

Thus, basic physiology of the heart, lung or kidney can be readily learned by one session of modeling, as contrasted with a series of animal preparations requiring several weeks of laboratory experiments on many animals. A comprehensive physiological model (HUMAN) is in use at Indiana Univ. (Coleman and Randall, 1983).

It must be understood that there are some situations for which there is no substitute for animal testing; the training of surgeons is an illustration. Pathology and histology require examination of both normal and abnormal organs and tissues, and these fields have not yet been simulated.

One potential drawback to simulations is that they may unintentionally train students to place unwarranted emphasis on data from instruments, rather than to observe the behavior and appearance of the patient or subject. (Walker, 1983).

At one medical school, the recent introduction of simulation programs for a cardiovascular circulatory model, the digestive system, and exposure to abnormal temperatures, have reduced the use of dogs in training of medical students by about 150 each year. Simultaneously, less time was required to perform the animal experiments. (Walker, 1983).

V. The Uses and Availability of Computer Models in Biology.

The usefulness of computer models in biology is based on their mathematical nature; until mathematical methods were used to explain or describe biological processes, only verbal or graphical descriptions were possible. Valid mathematical equations, however, represent concise explanations of how a system will be affected by changes in parameters such as time, volume, temperature, rate of flow, or pressure. These equations take the place of paragraphs of textual explanation, and represent a powerful shorthand.

Limitations on their utility are probably due to the lack of knowledge of all possible parameters which may play a role, however slight, in the melange of feedback mechanisms which constitute living systems. Basic research on all biological levels continues to provide new knowledge, and it is reasonable to expect continuing improvement of existing models, as well as development of models where no satisfactory model, as yet, has been developed.

Among the latter examples are tentative models of whole organism behavior, or of neurological input processing in the brain. Such models may be expected to be more complex than those previously discussed. As an example of the complex relationships encoutered in the brain, King, et al, (1984) report a model of dopamine neurodynamics which explains how both electrical stimulation of the nigrostriatal tract, and inhibition of dopamine firing, can result in increased dopamine systhesis at nerve terminals. Their model also predicts erratic swings in dopamine concentrations which are reminiscent of the mood swings and episodic occurrences of psychotics.

The NIH's Division of Research Resources has funded the establishment of a National Biomedical Simulation Resource at Duke University, to stimulate the growth of this field by providing free computer time and software assistance on-line. Academicians and noncommercial scientists may use this service for a period of 5 years, beginning in late 1984. Training sessions are planned to introduce biological scientists to the concepts of modeling, and special aid will be given to the development of computer programs (software assistance). (Kootsey, 1984).

It must be recognized that leaders in this field are unanimous in their evaluation that most biomedical experimentation will continue to require the use of living animal subjects for the forseeable future; the increasing utility of models will reduce the dependence on animals, but will not replace them completely.

Task 3. Computerized Predictive Structure-Activity Evaluation

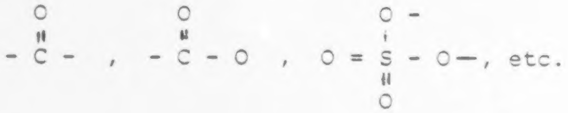
I. Basic Concepts of Structure-Activity Relationships (SAR)

A. Definition of SAR

If one can express the biological activity of a series of chemical substances in either numerical terms (such as ED_{50} or LD_{50}) or in quantal terms (such as "high", "medium", "low", "inactive"), then one can seek correlations between the biological activities and such properties, associated with the structures, as lipophilicity, electronic or steric parameters, or the presence of various substructural features in the molecules under study. Statistical methodologies are used to seek correlations, and to evaluate the significance of the correlations obtained.

B. Representations of "Structure"

1. Chemical structures may be considered to be composed of various substructural units, which represent combinations of small numbers of atoms. For example, all organic carboxylic acids contain the following combination of carbon, oxygen and hydrogen atoms: —COOH. One may use from 300 to some 2,000 or more combinations of the elements most often found in chemical substances to represent these descriptive units. The 300-level of substructural units are usually groups of from 1 to 5 or more atoms, representing functional groups as taught in chemistry; whereas the 2,000 + level are usually 1, 2, or 3 atoms, together with the other valence bonds, as exemplified:



$$-\overset{O}{\overset{\|}{C}}- \quad , \quad -\overset{O}{\overset{\|}{C}}-O \quad , \quad O=\underset{\underset{O}{\|}}{\overset{\overset{O-}{|}}{S}}-O—, \text{ etc.}$$

Of course, one could represent every individual atom as the ultimate substructural unit in a molecule, but experience has shown that combinations of atoms are far more useful for correlative purposes. (Lynch, et al, 1971).

2. In addition to the presence or absence of these descriptive fragments of molecules, their topological arrangements within a molecule are of great importance (topological arrangements refer to how these fragments are connected to each other). Some fragments can interact with each other when they are located in close proximity to each other. Most structure coding systems include both descriptive and topological codes.

3. The major advances in SAR in the past 25 years have used physical properties associated with the chemical structures for correlation with biological activities. (Martin, 1978) The physical properties most successfully employed are the partition coefficients, electronic parameters (such as the Hammett sigma constants), molar refractivity, and various types of steric constants. (Hansch and Leo, 1979)

In order to employ physical properties in a SAR study, one should work within a closely related series of chemical structures, with biological activities which were measured in a uniform manner. These SAR studies, known as "multiple parameter analyses", are often called "Hansch" correlations, after Prof. Corwin Hansch, who pioneered in this field 25 years ago.

C. Representations of "Activity"

1. "Continuous" data are those which one can measure with some known degree of accuracy, such as ED_{50} values (dose required to achieve a 50% effect). Most observable ED_{50} values can only be measured to about 0.8 to 1.2 times the average ED_{50} value (referred to as "confidence intervals"; e.g. $ED_{50} = 1.0 \pm 0.2$). For purposes of statistical validity, such data should have a range of at least 10-fold, and preferable, 100-fold, in the "continuous" ED_{50} values. ED_{50} data are usually expressed in mg/kg of test animal. For correlative purposes these are usually converted into moles/kg, "C", by dividing the ED_{50} in mg/kg by 1,000-times the molecular weight. Because the resulting numbers for C (molar concentration) are usually on the order of 0.00001, or 1×10^{-5}, these are usually expressed as the $\log_{10} \frac{1}{C}$, which in this case would be 5.0.

2. "Discrete" (or quantal) data are used when only rough estimates of biological activity are available, such as "*high, medium and low,*" or +, ++, +++; and even "active or inactive." Correlations attempted with data of this limited information content are usually less accurate than when continuous data are available, and discriminant equations, using probabilities of activity, are employed.

For SAR studies involving closely related compounds one should normally have at least five chemicals for each term in the resulting multiple parameter equation (Craig et. al., 1971) in order to ensure statistical significance of the equation. A frequent limitation on the application of SAR methods is the lack of sufficient data to permit significant analysis by these statistical methods. In SAR studies which involve diverse types of structures, one should employ at least 200 chemicals which have been tested (and evaluated) in the same biological test

D. Relationships Between "Structure" and "Activity"

1. The simplest relationship, which is often observed for limited data sets, is a linear relationship of the following type:

$$\log 1/C = a\ (\log P\ ) + b,$$

where "$\frac{1}{C}$" is the reciprocal of the equieffective molar concentration, "a" is a coefficient which is the slope of the straight line represented by plotting log $\frac{1}{C}$ against log P, "log P" is the log of the n-octanol/water partition coefficient, and "b" is the intercept of the straight line when log P = 0. Hansch has tabulated several hundreds of such equations, and finds most have slopes ("a") of from 0.7 to 1.2. (Martin, 1978).

2. A major problem with such straight line equations is the prediction that, on increasing the value of log P, an ever-increasing log $\frac{1}{C}$ value will result; experience has shown that there is usually a limit to biological activities. This has led to the development of parabolic equations when a large enough range of log P values are available in a given series. These

take the form of:

$$\log \frac{1}{C} = a\ (\log P) - b\ (\log P)^2 + c,$$

where c is now the intercept and "a" and "b" are coefficients which are determined by the regression (statistical method employed to solve for the equation). Such an equation predicts an optimum value of log P for which the biological activity will be at a maximum. Kubinyi developed a SAR "bilinear model" which does not assume a parabolic relationship between structure and activity. This model sometimes describes the experimental results more accurately than does the parabolic model. (Kubinyi, 1977)

3. An alternative method of SAR study is the "additivity" model, also called the Free-Wilson model. (Free and Wilson, 1964) Unlike the multiple parameter method, no physical properties are assumed to be involved, and only the biological activities of the chemicals tested and the substructural units contained in each chemical are used to generate de novo substituent coefficients for the substructural units represented. The method assumes that each time a given structural unit is present in a chemical, it will play a consistent role (positive or negative) towards the overall biological activity of that chemical (and for any other chemical in the series under study, which also contains that structural unit).

The additivity method has certain restrictions on its application, similar to the multiple parameter method, and the two methods can often provide similar guidance in development of active drugs (Craig and Hansch, 1973). The multiple parameter method is used far more frequently than the additivity method, even when both methods might be applicable, because it is more easily used, and also the resulting multiple parameter equation often provides insight into SAR which can be extrapolated beyond the limited series under study. This is usually not possible with results of the additivity analysis.

Application of knowledge gained in such QSAR studies can often be made to develop optimally active analogs in series of drugs or pesticides. The concept of an optimal value for the log P is often the key which can guide further synthesis and testing of biologically active compounds.

II. Development of Mathematical Models of Toxic Effects of Chemicals.

In the areas of toxicity, SAR studies have lagged behind the areas of drugs and pesticides, mostly because no one has been seeking more active carcinogens, mutagens, and teratogens. Early efforts to obtain SAR models for carcinogenicity involved the use of quantum chemical calculations for a series of substituted aromatic amines, but the resulting model did not permit extrapolation to other types of chemicals (Neely, 1975).

A. Rat Oral LD_{50} Models

The first paper by Free and Wilson reported the use of their method to correlate structures with LD_{50} data for a small set of closely related compounds. (Free and Wilson, 1964). In 1978 the first serious attempt was made to extend the additivity concept to 425 intentionally diverse chemicals for which rat oral LD_{50} data and substructures were available. (Enslein and Craig, 1978).

Hodes at NCI developed a statistical method which uses some 2,000 small substructure units to estimate antitumor activity, based on a training set of more than 10,000 diverse chemicals. (Hodes, 1982). His method uses probability statistics, and can handle many thousands of chemicals with ease. However, this method uses only the smaller substructure units, and does not include topological relationships among various substructures. His method has been extended by Tinker to develop a rat oral LD_{50} model (Tinker, 1982) and a mutagenesis model. An expanded rat oral LD_{50} model was developed from 1,851 chemicals and more than 300 possible substructures (Enslein, Lander, et al, 1983). Validation of the model was tested by estimating LD_{50} values for 908 compounds not used in development of the model. (Figure 2) Originally, 1,000 compounds were available for the validation study, but the model rejected 92 as having structural features not contained in the original 1,851 compounds used to develop the model. Further experience has shown about 10% of the compounds for which estimates were desired fall into this category; one cannot extrapolate beyond the scope of the structures included in the development of a model.

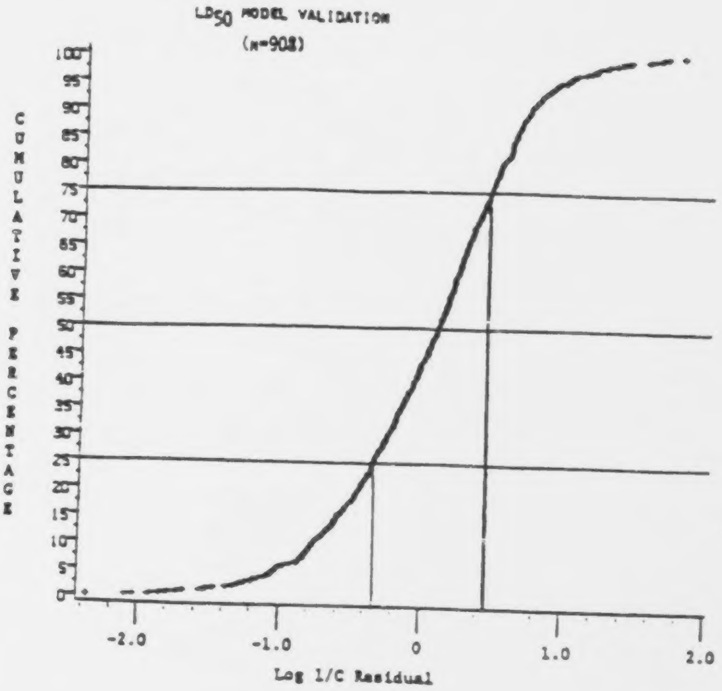
Nevertheless, about 90% of the 1,000 compounds submitted for LD_{50} estimation did fall within the scope of the structures represented in the model, and of these, 80% were estimated to within a factor of ± 6-fold (from 1/6 to 6 times the observed LD_{50}).

B. Carcinogenicity Models

At Penn State University, Peter Jurs and colleagues have published a series of discriminant models based on a combination of substructural features and physical properties such as molecular connectivities, surface area, molecular volume, and simple molecular orbital calculations. The models include carcinogenicity for polycyclic aromatic hydrocarbons (Yuan and Jurs, 1980), for nitrosoamines (Rose and Jurs, 1982), diverse carcinogens (Jurs, Chou, and Yuan, 1979), and aromatic amines (Yuta and Jurs, 1981).

These models have an internal consistency of from 75 to 97%; since they are discriminants, they basically estimate the probability that a given chemical is, or is not, a carcinogen or a mutagen.

The nitrosamines present a particular challenge to those who want to relate structure and carcinogenic activity. Hansch - type equations were developed by Singer, et al, (1977) and by Wishnok, et al, (1978) and Edelman, et al, (1980) for relatively small groups of nitrosamines. Jurs and colleagues studied nitrosamines and nitrosamides (Chou and Jurs, 1979),



Legend: On the abscissa are plotted the differences between the estimated and experimental values for the LD_{50} ("residuals"). A perfect agreement would have resulted in a vertical line at log 1/C residual = 0. The ordinate lists the cumulative percent of the 908 compounds.

Figure 2.

Representative Classification Results for *N*-nitroso Compounds

Analysis Method	% Correct (carcinogens, noncarcinogens)
Learning Machine Methods	
Linear Learning Machine	97.3 (99.1, 92.1)
Adaptive Least Squares	97.3 (99.2, 92.1)
Iterative Least Squares	94.7 (97.3, 86.8)
Discriminant Analysis Methods	
Bayes Linear (BAL)	88.7 (91.9, 78.9)
Bayes Quadratic (BAQ)	92.8 (95.5, 84.2)
Linear Discriminant Analysis (LDA)	88.7 (91.9, 78.9)
First Nearest Neighbor Analysis	81.3 (90.2, 55.3)

Table 5.

and later, Rose and Jurs studied a series of 150 nitrosamines. (Rose and Jurs, 1982) The latter work resulted in a model which used 22 descriptors and was shown to be internally consistent at the 90+% level in classification of carcinogenic nitrosamines. Eight of the 22 descriptors described electronic properties of these compounds, reflecting the importance of such properties for nitrosamines as carcinogens.

The consistency of the model developed for nitrosamine carcinogens is shown in Table 5.

A discriminant analysis model has been developed from the carcinogenic evaluations made for 343 diverse compounds by the International Agency for Research on Cancer, using the substructure approach (Craig and Enslein, 1982). The 343 compounds were either evaluated as animal or human carcinogens (223) or indefinite or non-carcinogens (120). The model accurately classified 93% of the compounds. The false negative rate was from 2 to 3%, and the false positive rate was 4%. Of the 343 compounds used in development of the model, about 10% were non-classifiable (this means that the discriminant for these compounds gave them about a 50% probability of being carcinogenic), although the IARC estimates were either "carcinogenic" or "indefinite or non-carcinogenic". (Table 6.)

A model which predicts the metabolic transformations, which often occur to form the ultimate carcinogen from precursors, is under development by Wipke, et al (1983).

C. Teratogenesis Model

A discriminant model for teratogenesis activity was developed from test results for 430 compounds, which were evaluated for teratogenic activity by a panel of experts. (Enslein, et al, 1983). The same substructure key system used for the carcinogenesis model was employed, (modified CROSSBOW code - see ref. Enslein et al, 1983), and 68 keys were found to contribute significantly to the model. The classification matrix for this model is shown in Table 7.

	CLASSIFICATION MATRIX Predictions by Model: Carcinogenicity:		
	Indefinite	Indeterminate	Definite
IARC "Indefinite"	93	14	13
IARC "Definite"	8	21	194

False Negatives = 8/308 = 2.6%

False Positives = 13/308 = 4.2%

Indeterminates = 35/343 = 10.2%

Classification Accuracy = 287/308 = 93.2%

Table 6.

	CLASSIFICATION MATRIX Predictions by Model: Teratogenicity:		
Panel Assessment:	Non-teratogenic	Indeterminate	Teratogenic
Non-teratogenic	133	46	15
Teratogenic	22	49	161

False Negatives = 22/331 = 6.6%

False Positives = 15/331 = 4.5%

Indeterminates = 95/426 = 22.3%

Classification Accuracy = 294/331 = 88.8%

Table 7.

D. Mutagenesis Models

Mutagenesis as a toxicity endpoint (especially the Ames test) has been studied by SAR methods by several workers, beginning with a multiple parameter analysis of 15 organoplatinum analogs in which Hansch and co-workers found an electron withdrawal parameter provided satisfactory correlation. (Hansch, et al 1980) Hopfinger and Potenzone studied a series of eighteen 1-phenyl-3, 3-dialkyltriazenes and found the 3-dimensional shape of these compounds to be a useful parameter for SAR studies of both Ames test activity and antitumor potency of this type of compound. (Hopfinger and Potenzone, 1982). Kier, et al, used molecular connectivity indices to correlate the Ames test data for 15 nitrosamines. (Kier, et al, 1978) Jurs and colleagues have begun studies of the Ames test data for a diverse set of chemicals evaluated as mutagens by the Gene-Tox program of EPA. (Jurs, et al, unpublished data) Tinker has used the substructural method of Hodes to develop a model for Ames test results from 100 diverse compounds. (Tinker, 1981) His model correctly estimated the mutagenecity of 88% of 34 unknown compounds to within ± one level of activity out of a range of eight levels. Craig and Enslein reported a discriminant analysis model which relates Ames test results for 416 diverse compounds to 48 substructural features. (Craig and Enslein, 1981) The classification index for this model is shown in Table 8.

E. Draize Test Models

The first effort to model the Draize skin irritation test is in progress. Enslein, et al, have used a subset of the total available data which contains 260 non-ring compounds. Skin irritation data have been correlated, using a new set of substructure descriptors, together with partition coefficients and molar refractivities, to generate a discriminant model. Preliminary results are encouraging that upon extension of this study to include ring compounds, it may prove possible to substitute the model for irritation studies of a high proportion of the, as yet, untested compounds. (Enslein, et al, private communication, 1984).

EMIC Classification:	CLASSIFICATION MATRIX Predictions by Model: Mutagenicity:		
	Non-mutagenic	Indeterminate	Mutagenic
Non-mutagenic	162	18	22
Mutagenic	17	16	181

False Negatives = 17/382 = 4.5%

False Positives = 22/382 = 5.8%

Indeterminates = 34/416 = 8.2%

Classification Accuracy = 343/382 = 89.8%

Table 8.

III. Current Status of Utility and Limitations of These Models.

The potential for reducing the use of animals in toxicity testing by substitution of these models is great, but must be tempered by realistic appraisal of the disadvantages as well as the advantages of these models.

The first consideration is that these are models which are developed by statistical methods from experimental data. Therefore, estimates made by use of such models have a higher standard error of estimate than that of a laboratory experimental value. This consideration must be balanced by the realization that animal toxicity studies also result in data which are burdened with standard errors of estimate.

Thus, when one speaks of an experimentally obtained LD_{50} value, implicit in the numerical value is a degree of uncertainty which arises from several factors, including variability of individual animals, slight differences in test protocol, and such factors as food, housing, lighting, and ventilation. The "actual" LD_{50} is an unknown value and will never be established with certainty. It is important to recognize that all toxicity testing is subject to this limitation; unfortunately, non-scientists are often unaware of this fact, and tend to consider the results of an animal toxicity test as absolute. When a duplicate toxicity test provides a different value, or even a different evaluation (e.g., carcinogenic or non-carcinogenic) the non-scientist tends to distrust the scientific process, rather than to recognize and accept its limitations.

Another consideration is the temptation to apply such models beyond the scope of the chemicals used to develop the models. Application of the models should be carried out by those familiar with the full range of structures represented in the model, and they must be careful not to extrapolate beyond the limits of the model. This is a special problem for those models developed from diverse types of chemicals; models developed from closely related chemicals are not as subject to this problem, because one is not usually tempted to extrapolate such results beyond the limited series studied.

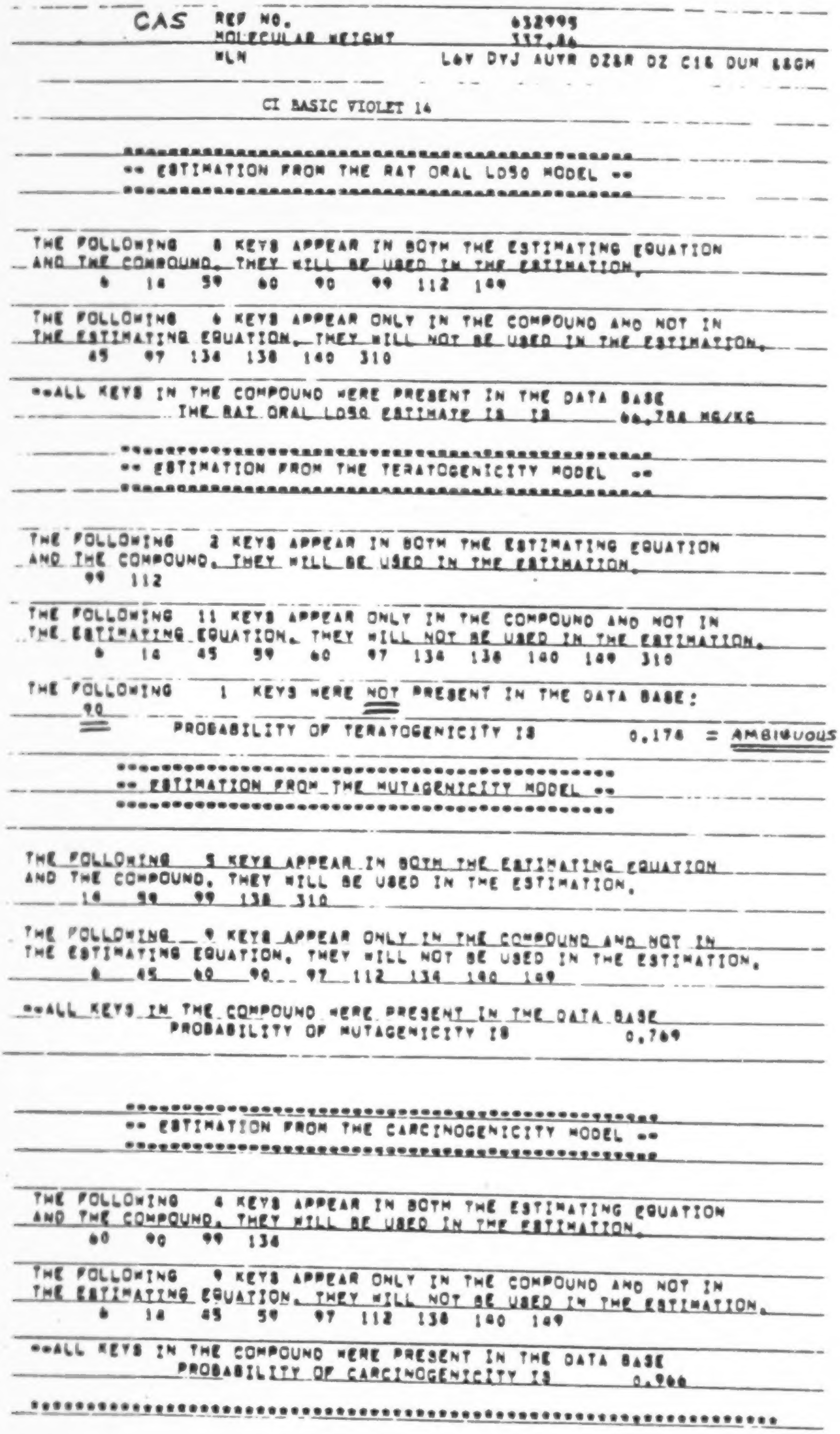
Countering these limitations are the major factors of economy, speed and conservation of animals and reduction in suffering of animals. The costs for an experimental rat LD_{50} value for one chemical may range from $500 to $1,000; this is the lowest price toxicity test at present. Application of the LD_{50} model may cost from 1/3 to 1/5 as much, depending on the number of estimates to be made simultaneously. The estimated costs for a carcinogenicity lifetime bioassay ranges upward of $500,000 for a single chemical, and usually requires 3 to 4 years. An estimate from the carcinogenicity models may cost about $300 and require about a day. These are the extremes; mutagenesis and teratogenesis test costs fall between these ranges.

The number of mice and rats used in LD_{50} testing can only be estimated, but is probably on the order of several million animals of each species every year. The potential economies, which could result by substituting the use of statistical models for a fair portion of these experiments are surely great. What is needed is a careful examination of the required degree of accuracy (i.e., what standard error of estimate is acceptable for an LD_{50} value?) in each case, to decide whether an estimate from a model or from a laboratory experiment would give the required degree of accuracy.

Application of these models was made by NTP in 1981-2 to assist in the selection of candidate compounds for animal testing. Tables 9 and 10 illustrate computer printout and examples of the type of qualitative guidance which these models can generate.

The major potential for use of mathematical models of toxicity is for setting priorities for large numbers of compounds which are candidates for toxicity tests, in order to reduce the numbers to be tested. For example, approximately 60,000 chemicals are contained in the TSCA Inventory List of EPA. More than 90% of the chemicals lack any toxicity data at all. Only some 800 have been tested for carcinogenicity, and fewer than 60 adequate new carcinogenicity tests are reported each year on a world-wide basis. Application of several of the available carcinogenesis models (Jurs, Enslein, et al) could be made for two or three thousand of the TSCA Inventory

EXAMPLE OF TOXICITY ESTIMATES (Health Designs, Inc.)



```
        CAS  REF NO.                        632995
             MOLECULAR WEIGHT               337.86
             WLN                    L6V DYJ AUYR DZ&R DZ C1& DUM &&GH

                     CI BASIC VIOLET 14

          ********************************************
          ** ESTIMATION FROM THE RAT ORAL LD50 MODEL **
          ********************************************

  THE FOLLOWING   8 KEYS APPEAR IN BOTH THE ESTIMATING EQUATION
  AND THE COMPOUND. THEY WILL BE USED IN THE ESTIMATION.
       6   14   59   60   90   99  112  149

  THE FOLLOWING   6 KEYS APPEAR ONLY IN THE COMPOUND AND NOT IN
  THE ESTIMATING EQUATION. THEY WILL NOT BE USED IN THE ESTIMATION.
      45   97  134  138  140  310

  **ALL KEYS IN THE COMPOUND WERE PRESENT IN THE DATA BASE
                THE RAT ORAL LD50 ESTIMATE IS  IS        66.784 MG/KG

          ***********************************************
          ** ESTIMATION FROM THE TERATOGENICITY MODEL  **
          ***********************************************

  THE FOLLOWING   2 KEYS APPEAR IN BOTH THE ESTIMATING EQUATION
  AND THE COMPOUND. THEY WILL BE USED IN THE ESTIMATION.
      99  112

  THE FOLLOWING  11 KEYS APPEAR ONLY IN THE COMPOUND AND NOT IN
  THE ESTIMATING EQUATION. THEY WILL NOT BE USED IN THE ESTIMATION.
       6   14   45   59   60   97  134  138  140  149  310

  THE FOLLOWING    1  KEYS WERE NOT PRESENT IN THE DATA BASE:
      90
                PROBABILITY OF TERATOGENICITY IS         0.174  = AMBIGUOUS

          ******************************************
          ** ESTIMATION FROM THE MUTAGENICITY MODEL **
          ******************************************

  THE FOLLOWING   5 KEYS APPEAR IN BOTH THE ESTIMATING EQUATION
  AND THE COMPOUND. THEY WILL BE USED IN THE ESTIMATION.
      14   59   99  138  310

  THE FOLLOWING   9 KEYS APPEAR ONLY IN THE COMPOUND AND NOT IN
  THE ESTIMATING EQUATION. THEY WILL NOT BE USED IN THE ESTIMATION.
       6   45   60   90   97  112  134  140  149

  **ALL KEYS IN THE COMPOUND WERE PRESENT IN THE DATA BASE
                PROBABILITY OF MUTAGENICITY IS           0.769


          ***********************************************
          ** ESTIMATION FROM THE CARCINOGENICITY MODEL **
          ***********************************************

  THE FOLLOWING   4 KEYS APPEAR IN BOTH THE ESTIMATING EQUATION
  AND THE COMPOUND. THEY WILL BE USED IN THE ESTIMATION.
      60   90   99  134

  THE FOLLOWING   9 KEYS APPEAR ONLY IN THE COMPOUND AND NOT IN
  THE ESTIMATING EQUATION. THEY WILL NOT BE USED IN THE ESTIMATION.
       6   14   45   59   97  112  138  140  149

  **ALL KEYS IN THE COMPOUND WERE PRESENT IN THE DATA BASE
                PROBABILITY OF CARCINOGENICITY IS        0.966

  ****************************************************************
```

Table 9.

EXAMPLES OF ESTIMATES OF TOXICITY MADE FOR NTP (1981-2) *

COMPOUND	CAS REG. NO.	ESTIMATED LD_{50} (mg/kg)	ESTIMATED PROBABILITY OF:		
			CARCINOGENESIS	MUTAGENESIS	TERATOGENESIS
QUINIDINE	56-54-2	489	negative	positive	negative
PAPAVERINE HCL	61-25-6	440	negative	negative	positive
TRIFLUTHYMIDINE	70-00-8	217	positive	equivocal	negative
CI PIGMENT RED NO. 8	6410-30-6	444	negative	positive	negative
CI BASIC VIOLET 14	632-99-5	67	positive	positive	(ambiguous estimate)
CI DIRECT BLUE 1	2610-05-1	34	positive	positive	positive
CI ACID BLUE 1	129-17-9	46	negative	negative	equivocal

* From internal NTP reports, 1981-2

Table 10.

List Chemicals for the cost of one animal lifetime carcinogenicity test. Those chemicals with highest estimated probabilities of being carcinogens could then be considered as priority candidates for animal testing.

Although it is not anticipated that estimated toxicity data could be used to meet regulatory requirements for safety considerations, there are two specific instances where such estimates might prove to be acceptable. The first is for the EPA's "Notification Requirements and Reportable Quantity Adjustments" (Federal Register 48, No. 102, 23552, May 25, 1983). EPA can base reporting requirements on LD_{50} values if no other data are available. It is possible that EPA and the chemical industry might agree to accept estimates of LD_{50} prepared by mathematical models in lieu of new experimental tests. The second is the existing DOT requirement for special labeling of chemicals with LD_{50} values of 50 mg/kg or less; again, estimates might suffice, or might be used to suggest which chemicals should be further tested to decide label requirements.

An additional utility for the models has been their use in identifying errors in data transcription which are often undetected until an abnormally high or low value is found to be at variance with the estimate from the model. This has been verified for 10 LD_{50} values which were reported in RTECS with errors of up to 1,000-fold, usually caused by confusion between "milli" and "micro" prefixes. (Tablell) These errors went unnoticed until the LD_{50} model estimates were found to disagree markedly with the printed RETCS values. (Craig, 1983)

Task 4: Hardware Considerations

I. Discussion

The dizzying pace which has been set by the computer industry in reducing hardware costs while increasing capabilities continues unabated. Hence, this is the most difficult area to be specific about in this report.

CHEMICAL*	CAS REG. NO.	LD_{50} (RTECS)	LD_{50} (LIT.)
n-Hexane	110-54-3	28.7 mg/kg	28,700 mg/kg
Methyl ethyl ketone	78-93-3	3.4 "	2,700 "
2-Fluoroethyl (1,1,2,2-tetrachloro)ethyl ether	339-65-1	(administered ip, not po; cannot be used in this study	
1,1-Dimethyl-3(α,α,α-trifluoro-m-tolyl)-urea	2164-17-2	89,000 mg/kg	8,900 mg/kg
1-Hydroxymethylcyclohexaneacetic acid, sodium salt	7009-49-6	20 "	36 "
4-Hydroxy-3-(1,2,3,4-tetrahydro-1-naphthyl)-coumarin	5836-29-3	16,500 "	16.5 "

* Chemicals whose estimates varied widely from values reported by RTECS in 1982.

Table 11.

The present minicomputers (costing from $50 - 250 K) perform better than large frame computers (costing 10 times as much) in use only 3 or 4 years ago. Even microcomputers (at 1/10 the cost of mini's) are fast catching up with the larger computers, and in a few more years should be able to perform some mathematical model development tasks. However, the assignment of substructural features and many of the matrix inversion steps require either a large mini or a main frame computer, both now and in the next few years. Thus at present, both for model development and use, at least a minicomputer is needed. (Enslein, private communication, 1984). For a centralized registry, a large computer (big mini or main frame) is needed, but it can be accessed by micro's or simple terminals.

II. Centralized Computer Registry of Biological Test Results

A. Mainframe Computer

1. Originally LADB was operated on a mainframe computer at Battelle Columbus Labs, via time-shared terminals.

2. A dedicated VAX (large minicomputer) took over the central distributive functions in Jan., 1981, resulting in improved response times for LADB.

B. Terminal Requirements

1. Any terminal which could access TYMNET could access LADB. (TYMNET is an online computer system which can be accessed by remote terminals via telephone nationwide.)

2. Any future system should take advantage of the great improvements in computer graphics terminals, which permit formatting of results in tabular, graphic or even pictorial form.

III. Attitudes of Research Funding Agencies Towards Computers

A. NIH

The Division of Research Grants (DRG), NIH, looks favorably on requests for computer costs in grant applications from academic or not-for-profit organizations. These "other direct costs" are often as high as 8 to 10% of the first year grant, and usually average 5 to 6% over a typical 3 year grant. DRG recognizes that computers are essential for modern biomedical research. (Private communication, Dr. John James, NIH-DRG, 1984)

B. NSF

The National Science Foundation also shares this favorable attitude toward grant requests for computer costs. However, both agencies emphasize the use of computers to support basic research, rather than on model development per se. Major uses include data recording from instruments, data refinement, statistical analysis, and word processing.

Emphasis in biological research on computational aspects, instrument recording, control and analysis, storage and retrieval, and in support of basic research, rather than on modeling, will probably remain for the indefinite future. This is due to two factors: first, the basic feeling, expressed by many research scientists, is that modeling will probably never completely replace their need for animal experimentation; second, the fundamental role assumed by computers. Like paper and pencil, they are involved at almost every level of research including literature searching, research planning, execution of experiments, data capture and analysis and in report preparation.

IV. Computer Requirements for Biological Modeling

A. SAAM and CONSAM (NIH Simulation and Modeling Programs)

1. Simulation and Arithmatic Modeling (SAAM) is a software package which was devised at NCI (NIH) over the last ten years, and is available for main frame computers and for several large mini's. It will support both graphic and non-graphic terminals, and is in wide use as an online service called CONSAM (Conversational Simulation and Modeling)

Many isolated programs have been developed for micro, mini and main frame computers, depending on availability, for generation and use of educational or research biological models. (See Task 2.)

The National Biomedical Simulation Resource at Duke University, sponsored by NIH-DRR, has a dedicated AD-10 digital simulation computer, which is connected to a VAX 11/750 host computer.

Software will be written in FORTRAN and C; and three high-level simulation languages will be available: MPS-10, CSSL-IV and ACSL. (Kootsey, 1984).

V. Computer and Data Requirements for Mathematical Modeling

A. Hardware Considerations

Development of the LD_{50} and other toxicity models by the method of Enslein at the level of 200 to 2,000 compounds required a large main frame computer. Application of these models (once developed) requires either a main frame or very large minicomputer. (Enslein, 1984). Development and application of models by the ADAPT system uses a large minicomputer, with a light pen or similar graphics terminal for input. (Jurs, 1984). Development and application of statistical models by the method of Hodes and Tinker requires use of main frame computers.

B. Software Considerations

Enslein's programs are written in Fortran with some routines in COBOL. Jurs, Hodes and Tinker's programs are written in Fortran.

C. Availability and Cost Considerations

Health Designs, Inc. (Rochester, NY) offers to provide estimates of LD_{50}, carcinogenicity, mutagenicity, and teratogenicity on a commercial basis. Costs range from about one-fourth those for LD_{50} or Ames tests, to one one-thousandth those for carcinogenicity lifetime bioassays. Health Designs uses Enslein's proprietary programs for all four endpoints; the programs are available for government use since they were partially supported by government funding. The National Institute for Occupational Safety and Health, Cincinnati, has mounted each of these programs for internal use by NIOSH. The programs are being used as originally developed. Health Designs, Inc., uses newer versions for each of these end points.

The ADAPT programs developed by Jurs, et al, have been mounted by EPA at Research Triangle Park, NC. They are being used to develop models for mutagenesis from the Gene-Tox program data.

The Hodes programs are available for general usage; Tinker at Eastman Kodak Co. (EK.Co.) has mounted those programs successfully, and has placed the EK.Co. version in the Quantum Chemistry Program Exchange, Indiana University, Bloomington, Indiana, for public distribution.

D. Data Considerations

The chemical structures of all compounds to be used in the development of a model (the training set) must be well defined. The corresponding biological test data should all have been obtained by the same test, and should be a carefully evaluated set, if at all possible.

Representation of substructural features can be made by use of various substructure coding systems, which can range from manually assigned fragment codes to augmented atom keys which can be computer-generated from connection tables. The ADAPT system generates keys such as surface area and molecular volume estimates, and several systems are available to estimate partition coefficients (n-octanol/water) for from 80 to 90% of the organic compounds of major interest. (Chou, JT and Jurs, PC, 1979; Leo and Hansch, 1984)

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